PESTICIDES IN ONTARIO: A CRITICAL ASSESSMENT OF POTENTIAL TOXICITY OF AGRICULTURAL PRODUCTS TO WILDLIFE, WITH CONSIDERATION FOR ENDOCRINE DISRUPTION

VOLUME 2: Triazine herbicides, Glyphosate, and Metolachlor

P. Takacs, P.A. Martin, J. Struger

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P. Takacs[1], P.A. Martin[1] and J. Struger[2]

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EXECUTIVE SUMMARY

This series of reports provides an in-depth review of the environmental toxicity, environmental concentrations and potential for endocrine disruption of a selection of pesticides used in Ontario. Our goal was to identify the potential for adverse effects to wildlife at environmentally relevant concentrations of the compounds extensively used in agricultural and urban landscapes.

There is growing evidence that many environmental pollutants have mechanisms of action which directly or indirectly disrupt/modulate the endocrine system. Such pollutants have been termed **endocrine disrupting chemicals (EDC)**; for the purposes of this assessment, the working definition of an EDC is:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

Pesticides or pesticide families were selected from 1993 and 1998 Ontario agricultural use estimates. The report is based on published information from the period 1978 to 1999. Compounds were chosen for review based on comparative use and toxicity class information. Only those compounds whose estimated use within Ontario exceeded 10 000 kg in 1993 were evaluated. Review results of the selected compounds are reported in several volumes. Volume 1 included the assessments of eight compounds within five pesticide families: an organochlorine insecticide (endosulfan), three ethylenebisdithiocarbamate fungicides (maneb, mancozeb, metiram), two dinitroaniline herbicides (trifluralin, pendimethalin), a halogenated hydrocarbon nematocide (1,3-dichloropropene), and an organophosphorus insecticide (azinphos-methyl). Pesticide mixtures are also discussed. Volume 2 examines the triazine herbicides, glyphosate, and metolachlor. As well, 2,4-dichlorophenoxyacetic acid (2,4-D), mecoprop and other pesticides used extensively in the urban landscape will be assessed in subsequent volumes.

Profiles for each pesticide or pesticide family include: a brief description of active ingredients; use patterns in Ontario; occurrence in surrounding natural environments; associated acute and chronic toxicity and potential for endocrine disruption; evaluation of risk to wildlife; and recommendations for further research and monitoring.

Triazine herbicides:

- Atrazine is frequently detected in surface waters of southern Ontario, often exceeding the Canadian Water Quality Guideline for the protection of aquatic life of 1.8 μg/L.
- There is strong evidence that endocrine disruption in aquatic organisms can occur at and below this guideline concentration.
- Atrazine can disrupt the neuroendocrine system at concentrations which occur in the environment. However, atrazine seems to be non-estrogenic in rodent and human studies, but does bind to alligator estrogen receptors. It is antiandrogenic and possesses several, not yet well understood, mechanisms of toxic action on the neuroendocrine system.

Glyphosate:

- There are insufficient data on environmental concentrations of glyphosate in surface water and sediments to properly evaluate biological exposure in agro-ecosystems of southern Ontario.
- The endocrine effects of glyphosate and surfactants used in formulations have yet to be evaluated thoroughly. It may be unlikely that direct estrogenic effects could occur from exposure to the active ingredient, but some non-ionic surfactants that may be part of glyphosate formulations, have been known to cause this response.

Metolachlor:

- Although metolachlor is frequently detected in surface waters of southern Ontario, levels only occasionally exceed the Canadian Water Quality Guideline for the protection of aquatic life.
- There is very little acute and chronic toxicity data for metolachlor.
- There is insufficient research examining endpoints relevant to endocrine disruption, upon which to base a conclusion regarding its impact on the endocrine system.

Priorities for further research and recommendations:

1. More exposure data are critical for the accuracy of provincial risk assessments. Well designed monitoring of the environmental concentrations of these pesticides (water, sediment, soil, animal tissues) should precede further assessments (i.e. keeping use patterns and chemical mixtures in mind).
2. Efforts should be made to investigate associated toxicity in indigenous species, particularly in those taxa that have traditionally been poorly represented (e.g. amphibians, reptiles, wild birds) with special attention paid to the most sensitive life stages.
3. Analytical methods used for glyphosate and its surfactants must be modified to improve detection limits to environmentally relevant concentrations.
4. Research on metolachlor should be conducted addressing endpoints of endocrine disruption.
5. Toxicity assessments of pesticide mixtures should be initiated, and combinations should reflect those most likely to occur in the environment (outlined in Volume 1, Table 7.2).
6. Best Management Practices, such as buffer strips and grassed swales, should be implemented to reduce herbicide inputs into surface waters in high risk and ecologically sensitive areas. Research on the efficacy of these methods when implemented should be conducted to determine further improvements to be made.
7. A revised Canadian Water Quality Guideline for the protection of aquatic life should be considered for atrazine because numerous effects in aquatic organisms have been reported around the guideline concentration. Consideration should be given to the issue of mixtures and additive effects of closely related compounds.

SOMMAIRE EXÉCUTIF

Cette série de rapports présente un examen en profondeur de la toxicité et des concentrations dans l'environnement de certains pesticides utilisés en Ontario, ainsi que de leurs effets perturbateurs possibles sur le système endocrinien. Notre objectif était de déterminer les effets nocifs possibles sur la faune, à des concentrations observées dans l'environnement, de composés largement utilisés dans les régions agricoles et urbaines.

Selon un nombre croissant d'études, un grand nombre de polluants de l'environnement utilisent des mécanismes qui, directement ou indirectement, perturbent le système endocrinien ou agissent sur lui: ce sont les substances chimiques perturbant le système endocrinien, définies comme ci-dessous aux fins de la présente

> *Agents exogènes interférant directement avec la synthèse, la sécrétion, le transport, l'activité de liaison ou l'élimination des hormones endogènes et des neurohormones, qui ont des effets physiologiques sur le système immunitaire,*

On a sélectionné des pesticides ou des familles de pesticides selon des estimations des utilisations agricoles en Ontario pour 1993 et 1998. Ce rapport est fondé sur des informations publiées de 1978 à 1999. On a choisi les composés à examiner d'après des informations sur leurs catégories relatives d'utilisation et de toxicité. On n'a évalué que les composés dont le volume d'utilisation estimé en Ontario dépassait les 10 000 kg en 1993, et les résultats de l'examen des composés sélectionnés sont présentés dans plusieurs volumes. Le volume 1 rassemble les évaluations de huit composés de cinq familles de pesticides : un insecticide organochloré (endosulfane), trois fongicides de type éthylène-bis-dithiocarbamate (maneb, mancozeb, métirame), deux herbicides de type dinitroaniline (trifluraline, pendiméthaline), un nématocide de type hydrocarbure halogéné (1,3-dichloropropène) et un insecticide organophosphoré (azinphos-méthyle). On examine aussi des mélanges de pesticides. Le volume 2 porte sur les herbicides de type triazine, le glyphosate et le métolachlore. De même, les volumes subséquents portent sur l'acide 2,4-dichlorophénoxyacétique (2,4-D), le mécoprop et d'autres pesticides

Les profils de chacun de ces pesticides ou famille de pesticides comportent notamment une brève description des matières actives; des tendances de leur utilisation en Ontario; de leur occurrence dans les milieux naturels avoisininants; de leur toxicité aiguë et chronique et de leur potentiel de perturbation du système endocrinien; ainsi que de leurs risques estimés pour la faune, et ils présentent des recommandations pour études

Herbicides de type triazine :

- On détecte souvent des résidus d'atrazine dans les eaux de surface du sud de l'Ontario, dépassant souvent la limite de 1,8 µg/L des Recommandations pour la qualité des eaux au Canada visant la protection de la vie aquatique.
- Beaucoup d'études indiquent qu'il peut y avoir des perturbations du système endocrinien chez les organismes aquatiques à des concentrations égales ou inférieures à cette limite.
- L'atrazine peut perturber le système neuroendocrinien aux concentrations mesurées dans l'environnement. Toutefois, elle semble avoir des effets non oestrogéniques chez les rongeurs et les humains, mais elle se lie aux récepteurs d'oestrogènes des alligators. Elle possède plusieurs mécanismes nocifs pour le système neuroendocrinien, encore mal compris à ce jour, et comporte des propriétés antiandrogènes.

Glyphosate :

- Les données disponibles sur les concentrations environnementales de glyphosate dans l'eau de surface et dans les sédiments sont insuffisantes pour qu'on puisse évaluer convenablement l'exposition biologique à cette substance dans les écosystèmes agricoles du sud de l'Ontario.
- On n'a pas encore bien évalué les effets sur le système endocrinien du glyphosate et des surfactants utilisés dans les formulations. Même s'il est peut probable que l'exposition aux ingrédients actifs occasionne des effets oestrogéniques directs, on sait que certains surfactants non ioniques potentiels des formulations de

Métolachlore :

- Même si on décèle souvent la présence de métolachlore dans les eaux de surface du sud de l'Ontario, ses concentrations ne dépassaient qu'occasionnellement la limite des Recommandations pour la qualité des eaux au Canada visant la protection de la vie aquatique.
- Il n'y a que très peu de données sur la toxicité aiguë et chronique du métolachlore.
- La recherche est insuffisante au niveau des paramètres pertinents pour l'étude de la perturbation du système endocrinien, ce qui empêche de tirer aucune conclusion utile concernant son impact sur le système endocrinien.

Priorités pour d'autres études et recommandations :

1. Il est essentiel d'obtenir plus de données quant à l'exposition afin d'obtenir des évaluations précises des risques au niveau de la province. Des études pertinentes de surveillance des concentrations de ces pesticides dans l'environnement (eau, sédiments, sol, tissus animaux) doivent précéder toute nouvelle évaluation (c'est-à-dire, qu'il faut tenir compte des profils des utilisations et des effets des mélanges de composés chimiques).
2. On doit tenter d'examiner les phénomènes de toxicité associés à ces pesticides chez les espèces indigènes, notamment chez des taxons habituellement mal représentés (par exemple, les amphibiens, les reptiles et les oiseaux sauvages), tout en accordant une attention particulière aux stades les plus sensibles de leur

3. On doit modifier les méthodes analytiques utilisées pour le glyphosate et ses surfactants afin d'améliorer les limites de détection pour les concentrations mesurées dans l'environnement.
4. On doit effectuer des recherches portant spécialement sur les paramètres pertinents de la perturbation du

5. On doit entreprendre des évaluations de la toxicité de mélanges de pesticides, et les combinaisons examinées devraient être celles qui sont les plus probables dans l'environnement (voir le sommaire dans le volume 1 et dans le tableau 7.2).
6. Dans les secteurs à milieu fragile et à risques écologiques élevés, on doit appliquer des pratiques de gestion optimales, comme la mise en place de bandes riveraines et de rigoles de drainage couvertes d'herbe, afin de réduire les apports d'herbicides dans les eaux de surface. La recherche sur l'efficacité de ces méthodes lorsqu'utilisées devrait être conduite de manière à déterminer si des améliorations supplémentaires doivent

7. Étant donné qu'on a signalé de nombreux effets chez les organismes aquatiques à des concentrations voisines de la limite recommandée, on devrait envisager la révision de la limite pour l'atrazine dans les Recommandations pour la qualité des eaux au Canada visant la protection de la vie aquatique. Il faudrait aussi tenir compte d'effets additifs (ou synergétiques) dus aux mélanges de composés étroitement apparentés.

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1.0 INTRODUCTION

1.1 PURPOSE OF REPORT

The purpose of this report is to provide an in-depth review of the environmental toxicity, environmental concentrations and potential for endocrine disruption of a selection of pesticides used in Ontario. Our particular goals were to identify adverse effects at environmentally relevant concentrations of these compounds and to prioritize compounds that may pose a risk to populations of non-target vertebrate and invertebrate wildlife. Further, based on our findings, we sought to point out data gaps in specific areas and to recommend directions for future research and monitoring.

1.2 OVERVIEW OF THE ENDOCRINE DISRUPTION ISSUE

The last decade has been one of intense focus on pollutants that may affect the function of the endocrine system in humans, fish and other wildlife. This report places some emphasis on potential endocrine disrupting properties of pesticides in use in Ontario. There has been a growing concern of contaminant-induced reproductive effects in wildlife and humans resident in the Great Lakes region [1-4].

Although many of the adverse physiological effects of chemicals affecting the neuroendocrine system have been known for over three decades, widespread attention to this issue only materialized in the early 1990s. Previous to this time, some of the effects that are now considered endocrine disruption were classified as sublethal or chronic toxicity and for the most part were not used for regulatory purposes, with the exception of some reproductive endpoints.

The events that brought endocrine disruption to the forefront of environmental toxicology were reports of reproductive abnormalities in American alligators [5,6,7], declining sperm counts in humans [8,9], induction of female specific protein production in males and the lack of gonad development in both sexes of fish [10,11,12], masculinization of marine snails [13], as well as endocrine toxicity and vaginal cancer in the daughters of diethylstilbestrol (DES) treated women [14,15].

The heightened interest in endocrine-related effects of pollution in recent years is evidenced by the vast number of articles and reviews published about various aspects of endocrine disruption (see [10,12,16-31]) and the numerous meetings and workshops that have been held [32,33].

In the U.S., congress passed the Food Quality Protection Act (1996) and the Safe Drinking Water Act (1996) which mandated the U.S. EPA to establish an advisory committee to assist in developing a screening and testing strategy for evaluating chemicals for their potential to cause effects via endocrine disruption. This strategy had to be developed and implemented within three years (i.e.: by 1999) [27,8]. A more extensive exploration of environmental endocrine disruption will be completed by the National Academy of Science.

The U.S. EPA's Science Policy Council's interim position [27] on endocrine disruptors states that:

> *The EPA is aware of and concerned about information indicating the possibility of adverse effects on human health and the environment associated with exposure to endocrine disrupters* and that *The agency does not consider endocrine disruption to be an adverse endpoint per se but rather to be a mode or mechanism of action potentially leading to other outcomes, for example carcinogenic, reproductive, or developmental effects routinely considered in reaching regulatory decisions.*

Although the above U.S. legislation focus primarily on human health consequences of endocrine active chemicals, the U.S. EPA has included wildlife health as an endpoint of concern. In this context, wildlife includes vertebrate and invertebrate species [12].

1.2.1 Definitions and Terms of Endocrine Disruption

The U.S. EPA [27] uses the terms "endocrine disrupting chemical (EDC)", "hormone disrupter", and "environmental endocrine disrupter" synonymously, and defines them as:

> *An exogenous agent that interferes with the synthesis, transport, secretion, binding-action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior.*

This definition, however, does not directly address the interactions of the nervous and endocrine systems and therefore allows for some ambiguity. The neuroendocrine system is any of the systems of dual control of certain activities in the body of some higher animals by nervous and hormonal stimulation. For example, the posterior pituitary gland and the medulla of the adrenal gland receive direct nervous stimulation to secrete their hormones, whereas the anterior pituitary gland is stimulated by releasing hormones from the hypothalamus.

Also, neurohormones are not specified in the above definition. Neurohormones are hormones that are produced not by an endocrine gland but by a specialized nerve cell and are secreted from nerve endings into the bloodstream or directly to the tissue or organ whose growth or function it controls. Examples of neurohormones are norepinephrine, vasopressin, insect juvenile hormone and ecdysone.

As stated by the U.S. EPA [27], of importance here is the concept that endocrine disrupting chemicals encompass more than just environmental estrogens and include any agents that adversely affect any aspect of the entire endocrine system. The endocrine system includes a number of central nervous system (CNS)-pituitary target organ feedback loops involved in regulating a multitude of bodily functions and maintaining homeostasis, and there are potentially several target sites through which environmental endocrine disruptors could act. Thus, impaired hormonal action could result as a consequence of altered hormone synthesis, release, clearance, or binding, regardless of the initial site of action. Effects may be acute, although are more likely to be delayed or not expressed for a period of time. Emphasis is placed on disruption of CNS-pituitary integration of hormonal and sexual behaviour, female and male reproductive system development and function, and thyroid function.

The European community [33] defines an EDC more broadly as:

> *An exogenous substance that causes adverse health effects in an intact organism or its progeny, secondary to changes in endocrine function.*

For the purposes of the current assessment the following definition of an EDC is used:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

1.2.2 Mechanisms of Action of Endocrine Disruption

In order to elucidate the mechanisms of action of endocrine active compounds, background information on endocrine physiology in the current context is provided by several recent reviews [9,14,21,25]. The modes of action of EACs can be categorized into five groups [14]; they may act as:

1. Alternate ligands that can bind in place of endogenous hormones.
2. Modulators of hormone metabolism.
3. Modifiers of signalling occurring subsequent to receptor-ligand binding.
4. Modulators of CNS components responsible for neuroendocrine regulation.
5. Target organ toxicants.

Other modes of action are also possible, including those not associated with the endocrine system. Some chemicals are active through several modes of action that may vary depending on the dose or other conditions. For example, several phytoestrogens bind to the estrogen receptor, inhibit the enzymatic conversion of estradiol to estrone and/or androgens to estrogens [16].

The estrogen and androgen receptors are nuclear receptors. As a result, the steroid hormones that bind them, as well as any estrogen or androgen mimic, must diffuse through the plasma membrane. Following steroid receptor binding, the activated receptor complex seeks out specific DNA motifs, termed hormone response elements, upstream of hormone responsive genes [19]. This results in mRNA production followed by protein expression.

Estrogen mimics act within the cell via three main mechanisms [19]:

1. Direct binding and activation of the estrogen receptor.
2. Binding to other nuclear receptors that then interact with an estrogen responsive element.
3. Through other receptor and/or signal transduction pathways that alter estrogen signalling.

Some endocrine disrupters, such as the fungicide vinclozolin, cause estrogenic effects although they are antiandrogens [27,16] while others may be estrogenic or antiestogenic depending on the cellular environment [19]. Other EDCs act on the aryl hydrocarbon receptor (AhR), including dioxins and PCBs [10].

Non-receptor mediated mechanisms of action of EDCs are exemplified by the phytoestrogen β-sitosterol and the organotin tributyltin (TBT). The former reduces the biosynthetic capacity of gonadal steroids while the latter inhibits the enzymatic conversion of androgens to estrogens.

The U.S. EPA [27] states that:

> *For virtually all toxic chemicals, the toxic action or stress imparted on an organism will likely be moderated by endocrine and immune processes that exist to maintain homeostasis. Because of this, it is difficult to elucidate whether a toxic action is directed specifically at an endocrine function or whether an endocrine process disruption is an indirect consequence of some other stress to the immune, nervous and /or reproductive system.*

The evaluation of endocrine disruption in wildlife is further complicated by critical windows during development and species specific endocrine function. The developing fetus is extremely sensitive to the hormonal environment in the uterus for example, and natural differences in hormone levels surrounding rat or mouse fetuses of only 10^{-12} M influence the timing of sexual development and the behaviour of the animal in adult life [10].

Similarly for fish, exposure to estrogen mimics during a narrow window spanning just 10 d either side of hatching can cause feminization of the subsequent fry, whereas at high doses the exposure period need only be two hours in some fish [10]. In birds, sexual differentiation is estrogen dependent but this is not the case in mammals. Consequently, birds may be more sensitive to EDCs than mammals during embryonic development.

1.3 PESTICIDE USE IN ONTARIO

Pesticides are applied to terrestrial and aquatic ecosystems within Ontario. The agriculture and forest industries rely heavily on pesticides, even though some integrated pest management programs have been implemented to reduce their use. Many green areas in urban environments are maintained with herbicides, and even certain water bodies are periodically directly sprayed with lampricides to control sea lamprey populations in the Great Lakes Basin.

Pesticides were first applied in Ontario in 1885, when acetoarsenite and copper sulphate were sprayed in apple orchards to control insect pests [35]. Since then, pesticides have gone through several transitions: from primarily inorganic and organometallic formulations to organochlorine insecticides, triazine herbicides, organophosphorous, carbamate, and later, pyrethroid insecticides; and, more recently, to sulfonyl urea and imidazolinone herbicides. Many of the earlier products, such as the organochlorine DDT, have been withdrawn from use in Canada and other developed countries because of unacceptable levels of toxicity or environmental persistence. In general, pesticides have become less persistent, lower in toxicity to non-target organisms with greater specificity, but with high efficiency at very low volumes.

Unlike most developed countries, Canada no longer requires pesticide manufacturers to provide public access to records of product sales, so there is no direct way to obtain accurate information on pesticide use. However, every five years since 1973, the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) has conducted a voluntary survey of Ontario farmers regarding pesticide use, in an effort to estimate this information. Products and quantities used for different crop types reported through the survey are used in conjunction with known areas of each crop type per county as attained through OMAFRA's annual statistical crop surveys [36] to extrapolate county-wide estimates of pesticide use. The two most recent surveys conducted were in 1993 [37] and 1998 [38]. In 1993, pesticide use estimates were based on 1800 farm surveys out of a provincial total of 61,432 farms (2.9%) whereas in 1998, only 1200 farms responded. Rapidly growing segments of the agricultural industry, such as greenhouse and nursery operations, were not surveyed prior to 1998. The 1998 survey included nursery, sod and ginseng farms but still did not include greenhouses. Treatments with surfactants and oils, livestock sprays and rodenticides were also not included.

In 1998 [38], the estimated total pesticide active ingredients that had been applied to field crops, fruits and vegetables that year, was 5,214,402 kg. The previous survey of 1993 [37] estimated a total pesticide use of 6,246,442 kg, representing a reduction in the volume of pesticide use by 16.5%. The 1993 and 1998 surveys show a general decline in pesticide use from earlier surveys. However, some compounds, including glyphosate and metolachlor have increased overall through time, and markedly since 1993.

Newer, high efficacy pesticides are being used more widely. These are applied at grams per ha instead of kg per ha, which is reflected in the reduced total volume of pesticides used. For example, the sulfonyl urea and imidazolinone herbicides have acute phytotoxicity to non-target plants at concentrations below the limits of analytical chemical detection [39,40]. The inability to detect these compounds in environmental samples is just one of the many factors that makes environmental risk assessment very challenging.

Pesticide use in urban areas is more poorly understood than use in agriculture. The Ontario Ministry of the Environment (MOE) surveys individuals receiving commercial pesticide applicators licenses every five years [37]. However, totals obtained through these surveys represent an unknown proportion of the total urban use of pesticides, given that most domestic use pesticides may be purchased and applied by unlicensed home owners for which there are no sales records. MOE did not conduct this survey in 1998.

Historical monitoring by Frank and associates [41-49] in the 1970s and 1980s documented pesticide concentrations in surface water, groundwater and Great Lakes tributaries in southern Ontario. More recent surface water quality monitoring has been focused on pesticides in agricultural ecosystems of concern (i.e.: fruit, tobacco, muck crops, row crops) and the urban environment [50-56].

1.4 ASSESSMENT METHODS

A subset of the pesticides used in Ontario agriculture [37] has been selected for critical evaluation in this series of reports. Selection was based on comparative use patterns and toxicity class data (Table 1.1). Only those compounds whose estimated annual use exceeded 10 000 kg [37] were evaluated. As part of the toxicity characterization, the inclusion or exclusion of each pesticide from the lists of potential endocrine disrupters drafted by the World Wildlife Fund (http://www.wwfcanada.org/hormone-disruptors/) and the U.S. EPA [27] was considered. With these use and toxicity guidelines, 12 compounds from eight pesticide families were selected for review (Table 1.1) in Volumes 1 and 2.

Each chapter profiles a pesticide or family of pesticides that was heavily applied in Ontario in 1993 and/or showed a relatively high level of toxicity to non-target organisms. Each chapter contains the following components:

- A basic description of the pesticide, including the nature of the active ingredient and any information on other surfactants or inert ingredients in applied formulations.
- Use patterns in Ontario agriculture.
- Occurrence in surrounding natural environments.
- Associated acute and chronic toxicity, and a critical assessment of the chemical's potential for endocrine disruption.
- A critical evaluation of risk posed to Ontario wildlife by continued use.
- Recommendations for research and monitoring relevant to Ontario environments.

For Volume 1, the use pattern maps were derived using 1993 data from Hunter and McGee [37] for Ontario, and from Gianessi and Anderson [57] for U.S. states in the Great Lakes Basin. In Volume 2, these maps were derived using data from the 1998 census [38] for Ontario only. Assessment of toxicity was conducted by examining peer-reviewed publications and government reviews of pesticides and endocrine disruption, and by systematically searching for all combinations of active ingredient and/or chemical family names in the Life Sciences Index, Environment Abstracts, Water Resources Abstracts, and Pollution Abstracts (1978 - 1999).

1.5 REALISTIC ASSESSMENT OF RISK: MIXTURES, UNKNOWN INGREDIENTS, SURROGATE DOSES, AND HISTORICAL PERSISTENCE

Before continuing with independent evaluations of the selected pesticides, the influence of mixtures, surfactants, and historical loads of banned compounds on the assessment of overall risk should be briefly addressed. A more detailed discussion of pesticide mixtures present in Ontario may be found in chapter 7 of Volume 1.

A comprehensive review of pesticide use in England, Scotland and Wales found that 59 different combinations of two or more active ingredients were applied to 30 000 ha or more, and that an average of two mixtures per crop were sprayed simultaneously [58]. Spray recommendations published by OMAFRA for local fruit, vegetable, and flower (greenhouse) production [59-62] also refer frequently to co-formulations and mixtures of formulations, suggesting there is also widespread use of pesticide combinations in this country. Since most toxicity testing is completed with one treatment ingredient at a time, the toxic behaviour of mixtures is largely unknown [58].

Pesticides also contain what have traditionally been referred to as "inert ingredients" which may be surfactants, solvents, emulsifiers, adjuvants, stabilizers, etc. The use of the term "inert" has been criticized [63], since breakdown products of some of these components have been shown to disrupt the endocrine system, for example, the breakdown of ethoxylated nonylphenols to nonylphenol. Typically, only information on the active ingredient is divulged by the manufacturer, with information on other ingredients considered proprietary. Sometimes limited data are available (e.g. the diazinon formulation, Basudin 500EC contains a petroleum derivative solvent), usually because legislation demands that all hazardous ingredients be listed on material safety data sheets. In other instances, impurities may be surmised by knowing something of the manufacturing process; for example, when organochlorines like endosulfan are synthesized, dioxins, furans and hexachlorobenzene are sometimes present in small quantities in the commercial formulation [1, 63]. Also, alkyl phenols (including nonylphenols) are commonly used as surfactants for insoluble pesticides. The small quantities of inert ingredients and impurities in formulations likely preclude acutely toxic effects, but their presence may contribute to additivity or synergy, particularly with responses of the endocrine system. Polycyclic aromatic hydrocarbons (PAHs) from petroleum solvents, dioxins, furans, hexachlorobenzene and nonylphenol have all been implicated as potential endocrine disrupters [27].

In addition to these factors that complicate assessments of toxicity, there are others that complicate assessments of exposure. Water concentrations are generally a poor surrogate for internal dose of toxicant. They may be too low to be detected by standard analytical methods, yet be bioconcentrated in sensitive species over time. These and other factors make chemical concentrations in abiotic media a poor choice for pesticide risk assessment. Body residues generally provide a better surrogate of the dose at the site of toxic action. A body residue known to be associated with a particular biological response (e.g. acute lethality) is termed a critical body residue. By comparing critical body residues to known tissue concentrations it may be possible to improve determinations of environmental risk for those compounds. Existing tissue and sediment analyses have verified that several banned chlorinated compounds persist in Ontario environments [64-68]. These chemicals include DDT, mirex, dieldrin, and polychlorinated biphenyls (PCBs). Their presence in the environment, in combination with mixtures of in-use pesticides, might contribute to triggers of toxic response pathways in wildlife. By nature of their persistence, these chemicals are more likely to affect species higher in the food chain. The persistent organochlorines are also those which are most frequently discussed as potential or known endocrine disrupters [1,27].

Table 1.1 A selection of agricultural pesticides used in Ontario. Compounds that are underlined were selected for review in Volume 1; those in bold font are reviewed in Volume 2.

	1993 Use (kg ai.)[a]	1998 Use (kg ai.)[a]	% Change 1993-98	Potential Endocrine Disrupter[b]	Soil DT50 (days)	Solubility (mg/L)[c]	Mammalian Toxicity[c,d]	Fish LC_{50} (mg/L)[c,e]
Metolachlor (H)	1 327 315	1 376 570	+4		20	488	III	3.9
Atrazine (H)	589 852	598 206	+1	X*	16-77	33	III	4.5-11
Glyphosate (H)	414 821	647 494	+56		3-174	11 600	III	86
Dichloropropene (N)	410 512[f]	177 000	-57		2-17	2 000	III	3.9
Dicamba (H)	255 528	205 522	-20		<14	6 500	III	135
Metribuzin (H)	254 276	71 761	-72	X	-	1050	III	76
2,4-D[g] (H)	222 746	145 720	-35		<7	311	II	>100
Cyanazine (H)	215 480	49 038	-77	X*	14	171	II	16[h]
MCPA (H)	161 605[i]	119 700	-26		<7	734	III	232
Mancozeb (F)	155 463	156 269	+1	X	6-15	6.2	III	2.2
Captan (F)	151 468	101 276	-33		1	3.3	III	0.072[j]
Chlorothalonil (F)	115 613	120 751	+5	X*	5-36	0.81	III	0.049
EPTC[k] (H)	113 030	46 312	-59		-	375	II	19
Trifluralin (H)	83 945	23 250	-72	X	57-126	0.184	III	0.01-0.04
Sulphur (F)	72 338	55 670	-23		-	insoluble	III	non-toxic
Azinphos-methyl (I)	71 983	14 120	-80		weeks	28	Ib	0.02
Metiram (F)	57 230	131 113	+129	X	-	insoluble	III	1.1
Pendimethalin (H)	51 414	115 687	+125	X[†]	90-120	0.3	III	0.14
Maneb (F)	49 440	1 873	-96	X	25	insoluble	III	1.8[l]
Bromoxynil (H)	45 317	58 854	+30	X*	10	130	II	0.46[m]
Terbufos (I)	38 282	1 297	-97		9-27	4.5	Ia	0.01
Endosulfan (I)	25 930	6 909	-73	X	30-70	0.32	II	0.002[n]
Carbaryl (I)	16 882	15 334	-9	X	7-28	20	II	1.3
Carbofuran (I)	15 213	2 652	-83	X	30-60	320-350	Ib	22-29
Cypermethrin (I)	12 780	6 310	-51	X	5	0.004	II	0.001

[a] Use estimate based on 1800 farm surveys (1993) [37] and 1200 surveys (1998) [38]. [b] X indicates potential for endocrine disruption as per the U.S. EPA [27] and the World Wildlife Fund (http://www.wwfcanada.org/hormone-disruptors/); * signifies that it was only listed by WWF, whereas † signifies that it was only listed by the U.S. EPA. [c] source: Tomlin [69]; [d] Toxicity class based on World Health Organization (WHO) classification; Ia = extremely hazardous, Ib = highly hazardous, II = moderately hazardous, III = slightly hazardous. [e] Rainbow trout 96-h LC_{50} unless otherwise indicated. [f] the sum of 1,3-dichloropropene and dichloropropene. [g] the sum of amines and butyl esters of 2,4-D ([2,4-dichlorophenoxy]acetic acid). [h] Fathead minnow 96-h LC_{50}. [i] the sum of MCPA ([4-chloro-2-methylphenoxy]acetic acid) and MCPA/MCPB. [j] Bluegill sunfish 96-h LC_{50}. [k] EPTC = dipropylcarbamothioic acid S-ethyl ester = dipropylthiocarbamic acid S-ethyl ester. [l] Carp 96-h LC_{50}. [m] Goldfish 48-h LC_{50}. [n] Golden orfe 96-h LC_{50}. (H)-herbicide, (I)-insecticide, (F)-fungicide, (N)-nematocide, (-)-not available.

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2.0 THE TRIAZINE HERBICIDES: ATRAZINE, CYANAZINE AND SIMAZINE

The triazines replaced 2,4-D as the dominant herbicides in corn production in 1960, the year they were registered for use, partly because 2,4-D had inherent problems with crop injury and movement off the treated area [1,2]. The introduction of the triazines provided corn growers with a very economical herbicide that had long residual activity, high crop tolerance and a broad spectrum of weed control. By the 1970s over two-thirds of U.S. corn acreage was treated with atrazine.

The triazines are the second largest selling group of herbicide, with a 22% market share, and are preceded by the amide herbicides with 30% market share in the competitive U.S. herbicide market [1]. Annual application of atrazine in the U.S. during the mid 1980s was 34-36.4 million kg active ingredient per year. Annual use rate has declined since then in the U.S. and Canada, but has remained relatively constant throughout the 1990s [2,3].

North American surface water and ground water surveys initiated in the 1970s and 1980s showed that atrazine residues were detected over a wide geographic scale, and often exceeded the maximum contaminant level (MCL) of 3 µg/L [4,5,6,7]. In 1990-92, voluntary atrazine risk reduction measures were taken by Ciba Plant Protection (current registrant is Syngenta Crop Protection Inc.) [8]. These measures included label amendments with a reduction in application rates, and the classification of atrazine as a restricted use pesticide, to be used only by certified applicators or under such supervision. Some uses were removed from the label and the agricultural community was required to establish buffer zones or setbacks around aquatic habitats.

In November 1994, the U.S. EPA began a special review of atrazine, cyanazine and simazine, prompted by concerns of cancer risks to humans, through food, water and occupational exposures [7, 8]. Although ecological effects were not a trigger for the action, the agency was concerned about the widespread residues of the triazine herbicides and their potential ecological effects [7]. Ciba-Geigy requested that the Institute of Wildlife and Environmental Toxicology at Clemson University form a multidisciplinary expert panel to conduct an aquatic ecological risk assessment of atrazine [7].

Cyanazine has been withdrawn from the special review because the registrant agreed, in 1995, to voluntarily cancel all registrations and phase out the use of this herbicide in the U.S. Production of cyanazine has ceased for the U.S. market and it may no longer be used after 2002 [8]. A similar document is to follow for simazine.

In 1991, the EPA published the pesticide ground water strategy [8], which called for states to establish Pesticide Management Plans (PMPs). In 1996, a proposed rule was published that would prohibit the use of atrazine, cyanazine and simazine in states that did not have a PMP approved by the EPA. Some states, such as Wisconsin and Louisiana, have already taken steps toward limiting surface and groundwater contamination by atrazine, above and beyond federal regulations. In Wisconsin, the *Atrazine Rule* was adopted in 1988 and has been reviewed and amended annually. This rule set application rates below federal rates and restricts the area of use for atrazine. In Wisconsin, the enforcement standard is 3.5 µg/L, and the preventive action limit (PAL) is 0.35 µg/L of atrazine in groundwater. In Louisiana, the department of environmental quality has been conducting an exposure assessment in the Terrebonne Basin and is planning to limit the application of atrazine to certain crops if the 1999 annual average residue level exceeds the maximum allowable level of 3 µg/L, and to continue with restrictions until this goal is achieved.

Some European countries, including Norway and Germany, suspended the use of atrazine. For example, in Germany, atrazine use was banned in 1991 because ground water concentrations exceeded the allowable level of 0.1 µg/L [9].

Triazines have a short half-life in plants and animals but are quite persistent in some environments. They readily move in runoff and leach into groundwater via non-equilibrium sorption, where they are even more persistent with a DT50 of up to several years [10]. Although reductions in volume of use have taken place in recent years, atrazine is still the second most heavily used herbicide in the U.S. [1] and third in Ontario, Canada [3,11].

The World Wildlife Fund [12] listed atrazine and cyanazine as having potential for endocrine disruption. Given the high volume of use, high level of toxicity to primary producers, and long persistence in the environment, triazine herbicides were included in this report for further evaluation regarding wildlife toxicity, with special attention to endocrine disruption.

2.1 DESCRIPTION AND USE

2.1.1 Atrazine

Atrazine (2-chloro-4-ethylamino-6-iso-propylanmin-s-triazine) was registered by Ciba-Geigy in 1958. It is a selective herbicide with long residual activity in soil. Atrazine controls a wide range of grassy and broad-leafed weeds such as mustards, purslane, ragweed, smart weed, lady's thumb, wild buckwheat, lamb's-quarters, pigweed and volunteer clover. Some populations of ragweed and pigweed are now resistant to atrazine. Atrazine is used on crops such as corn, sorghum, sugarcane, pineapples, and nursery conifers, as well as forestry and lawn care applications [2,6,7]. The major use, however, is in corn production: corn accounted for 83% and 99% of the total volume applied in the U.S. and Canada, respectively. Domestic uses in Canada have included algae control in aquariums, ornamental ponds, and application as a soil sterilant around driveways, fences, and other non-crop lands [6]. Depending on the crop, atrazine may be applied preemergence or postemergence to control broad leaf and grassy weeds. This herbicide is primarily applied preemergence, as a water-diluted spray or tank mixed with liquid fertilizer, by ground equipment [7]. Usually a single treatment is made in the spring with or without oil, before annual weeds reach 4 cm. Rates of 1-4 kg ai/ha (ai = active ingredient) have been recommended in the past although label restrictions have reduced the maximum total pre- and postemergence use rates to 2.2-2.8 kg ai/ha by 1993 [7]. Actual use rates are estimated to be in the 1.6 kg ai/ha range. Current Canadian application rates in corn are 1.1-1.6 kg ai/ha [13].

Although the primary use for atrazine in Ontario is field and sweet corn, it is also registered for use on low bush blueberries and canola [13] (Table 2.1). Table 2.2 lists physical and chemical properties of the triazines. In Ontario, atrazine usage has decreased from 1719 tonnes to 585 tonnes active ingredient between 1983 and 1993, but has remained relatively steady between 1993-98 (573 tonnes in 1998) [11: see Figure 2.1].

Atrazine is sold in liquid, wettable powder, emulsion, flowable suspension and granular formulations. In addition, there are five formulated mixtures of atrazine and other herbicides used in Ontario. Atrazine may also be tank mixed by the applicator, with several other annual grass and broad leaf herbicides such as cyanazine to increase the spectrum of weed control in corn. Several blends of oil and surfactants are available for use with atrazine to improve their coverage of weeds. Compounds included in this category are: 1% emulsifier + oil, 17% surfactant + 83% mineral oil, surfactants containing 20-100% anionic polyethoxylated compounds [13]. The oil may be a mixture applied at 10-17 L/ha.

2.1.2 Cyanazine

Cyanazine (2-[(4-chloro-6-(ethylamino)-s-triazin-2-yl)amino]-2-methylpropionitrile) is one of the newer s-chloro triazine herbicides introduced by Shell Chemical Corporation in 1970. It is currently registered by DuPont DeNemours Co. and is also produced by Griffin Corporation. The registrants have agreed with the U.S. EPA to phase out the production and use of cyanazine in the U.S. market by the end of 2002 [8]. Cyanazine is a selective herbicide used for control of broadleaf and grass weeds in corn, canola, sorghum, soybean, potatoes and peas. In Ontario, it is registered for use on corn and canola [14,16,] (Table 2.1).

Cyanazine sensitive weeds include: annual nightshades, lady's thumb, shepard's purse, wild buckwheat, crabgrass, green and yellow foxtail, pigweed, panicum and others. Application methods include preplant incorporated in corn but only in mixture with other herbicides; also as preemergence in corn. Cyanazine controls many of the same broadleaf weeds as atrazine but is applied at 50-100% higher rates (e.g. 2-3 kg ai/ha) (Table 2.1).

Cyanazine is formulated as a mixture with several herbicides and may also be tank mixed at application. Its trade name is Bladex, and it is available in 480 g/L liquid suspension and as a 90% dry flowable formulation in Ontario. It can be formulated as a mix with atrazine, dicamba, dimethenamid, imazethapyr, metolachlor or pendimethalin [14].

The use of this herbicide in Ontario has steadily declined since 1978. Application to corn generally accounted for 99% of the amount used. A total of 513 metric tonnes were used in 1978, by 1988 the amount used was 227 tonnes and by 1993, 215 tonnes [3,16] representing a reduction of 58% between 1978-1993. By 1998, cyanazine use had further dropped to 49 tonnes [11], representing an additional decrease of 77% (Figure 2.2). This trend seems to be in step with the cancellation of the registrations of cyanazine in the U.S. Nevertheless, there has been no initiative in Canada to follow the U.S. cancellations.

Figure 2.1 Atrazine use in the Great Lakes Basin.

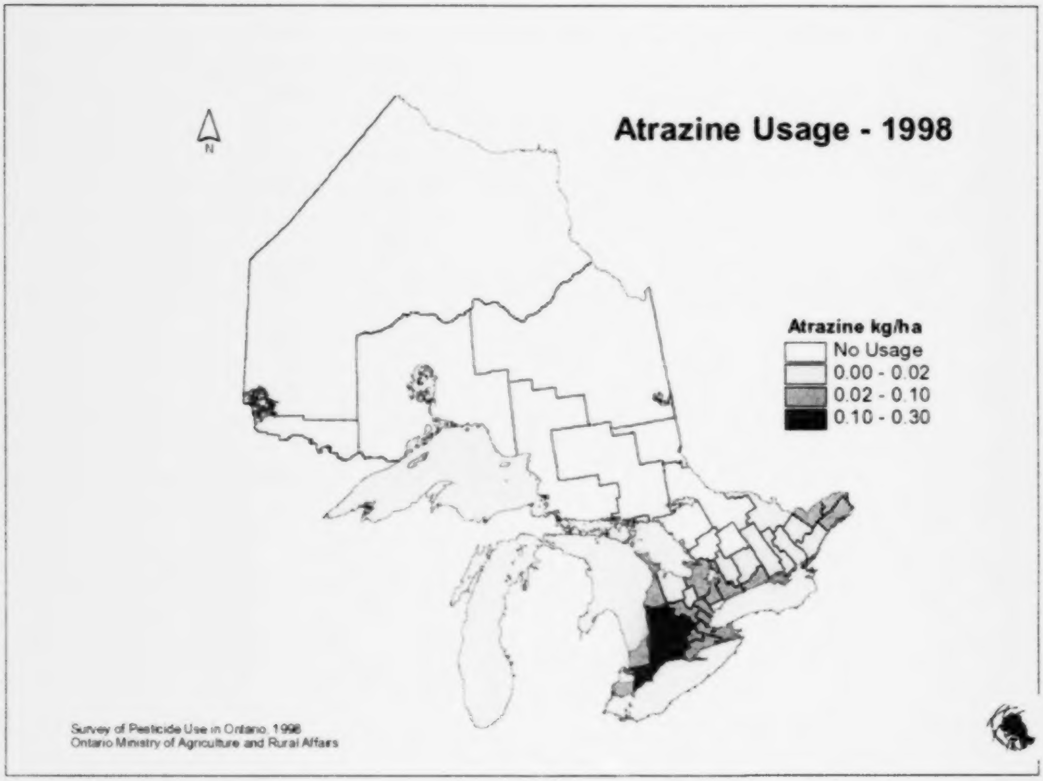
Atrazine Usage - 1998
N
Atrazine kg/ha
No Usage
0.00 - 0.02
0.02 - 0.10
0.10 - 0.30

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Table 2.1 Recommended applications of atrazine, cyanazine and simazine in Ontario agriculture, 1997-1999 [13,14].

Crop protected	**Herbicide formulation**	**Weeds controlled**	**Application rate (active ingredient for non-liquid formulations)**
Atrazine			
Field corn	Aatrex nine-O	Annual grasses and broad leaf weeds: mustards, purslane, ragweed, smart weed, lady's thumb, wild buckwheat, lamb's-quarters, pigweed and voluntee clover.	1.1-1.7 kg ai/ha
Sweet corn	Atrazine 480 Marksman (atrazine + dicamba)	see above	2.1-3.1 L/ha 4.5 L/ha
Cyanazine			
Field corn	Bladex liquid	Annual grasses and broad leaf weeds: annual nightshades, lady's thumb, shepard's purse, wild buckwheat, crabgrass, green and yellow foxtail, pigweed, panicum and others.	3.5-5.5 L/ha
Sweet corn	Bladex 90 DF	see above	2-3 kg ai/ha
Simazine			
Apples	Simazine 480	Annual grasses and broad leaf weeds: annual nightshades, lady's thumb, shepard's purse, wild buckwheat, crabgrass, green and yellow foxtail, pigweed, panicum and others.	1.6-4 kg ai/ha
Apricots	Terraklene (simazine + paraquat)	see above	2.8-5.5 kg ai/ha
Grapes	Simazine 80 W Simazine 480	see above	0.5-1 kg ai/ha 7.5-9.4 L/ha
Pears	Princep Nine-T	see above	1.25-2.5 kg ai/ha

Table 2.2 Physical and chemical properties of three triazine herbicides [2,6,15]

	Atrazine	**Cyanazine**	**Simazine**
CAS number	1912-24-9	21725-46-2	122-34-9
Chemical formula	$C_8H_{14}ClN_5$	$C_9H_{13}ClN_6$	$C_7H_{12}ClN_5$
Molecular weight	215.7	240.7	201.7
Melting point	173 °C	166-67 °C	225-227 °C
Henry's law constant	2.45×10^{-7} 3.04×10^{-9} atm m^3/mol @ 20°C		
Vapour pressure	3.0×10^{-7} mmHg @ 20 °C 6.9×10^{-6} mmHg @ 25° C 1.4×10^{-6} mmHg @ 30° C	1.6×10^{-9} mmHg @ 20 °C	6×10^{-9} mmHg @ 20°C
Solubility water methanol	28 mg/L @ 20°C, 33 mg/L @ 25°C, 70 mg/L 25°C 18 000 mg/L	171 mg/L @ 25°C 195 g/L	5 mg/L @ 20°C, 40 g/L
Log K_{ow}	2.3-2.8	1.8-2.2	1.9-2.2
Log K_{oc}	1.95-2.71		

2.1.3 Simazine

Simazine (2-chloro-4,6-bis(ethylamin)-3,5-triazine) was introduced by J.R. Geigy in 1956. It is a preemergence herbicide used to control broadleaf and grassy weeds in deep rooted crops such as fruit trees and bushes. It is also used on corn and a wide range of ornamental plants. Simazine was registered in Canada in 1963 [17]. It is currently registered in Ontario for use in corn, asparagus, apples, cherries, grapes, Christmas trees, nursery stock, and a wide range of other fruit crops [14]. Simazine is mainly absorbed by the roots; little foliar penetration occurs. It is translocated in the xylem to the apical meristem. It may be preplant incorporated or applied preemergence.

Simazine controls a similar range of weeds as do atrazine and cyanazine [13] (Table 2.1). Non-crop uses of simazine include industrial applications, airports, right of ways, aquatic weed control in ditches, fish hatcheries etc. [17]. Formulations registered in Ontario include Princep Nine-T, Simadex, Simazine, Simazine 480, and Simazine 80 WP. Simazine has been formulated as a mixture with other herbicides; however, only Terraklene (simazine + paraquat) is registered for use in Ontario [14]. It can also be tank mixed with other herbicides. Total use of simazine declined by 61% between 1978 and 1993 [3,17] but increased again by 54% between 1993-1998 (4900 kg [11]: see Figure 2.3). Based on the 1993 data, the amount of simazine used in Ontario was 0.5% of the volume of atrazine used (approximately 3000 kg). About 78% of simazine was applied on fruit crops and the remainder was used on vegetables.

Figure 2.2 Cyanazine use in the Great Lakes Basin.

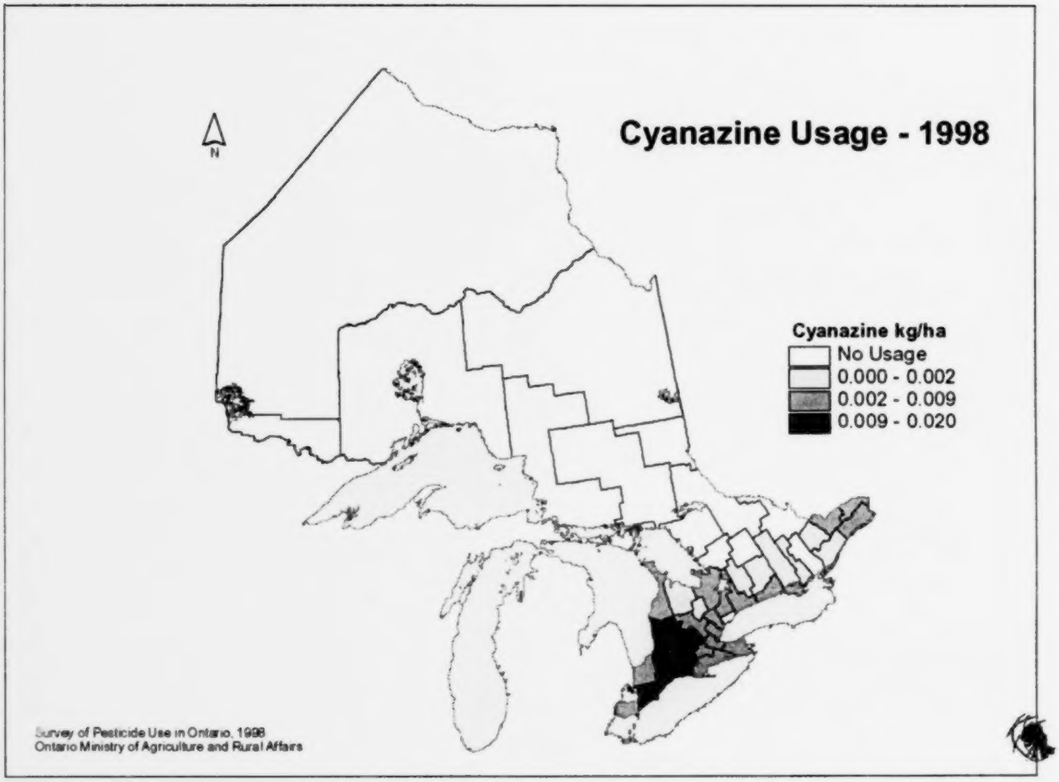
N
Cyanazine Usage - 1998
Cyanazine kg/ha
No Usage
0.000 - 0.002
0.002 - 0.009
0.009 - 0.020

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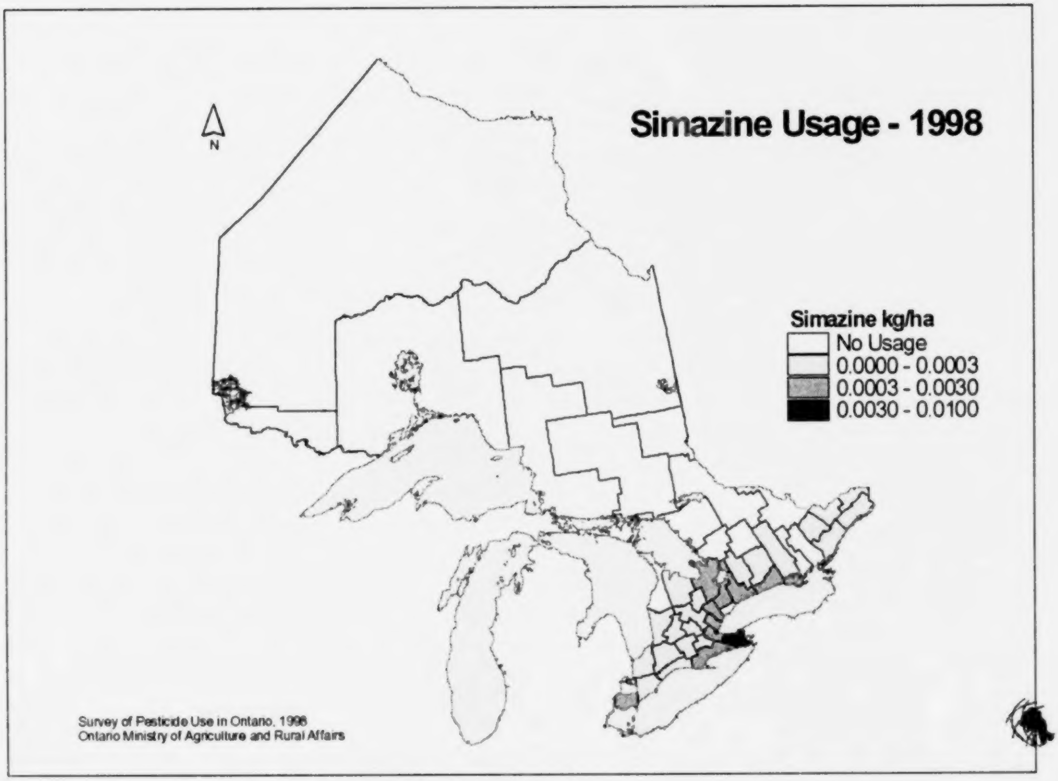
Figure 2.3 Simazine use in the Great Lakes Basin.


N
Simazine Usage - 1998
Simazine kg/ha
No Usage
0.0000 - 0.0003
0.0003 - 0.0030
0.0030 - 0.0100

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2.2 ENVIRONMENTAL CONCENTRATIONS

2.2.1 Atrazine

2.2.1.1 *Soil*

Processes governing the fate of atrazine in the environment include hydrolysis, adsorption, microbial degradation and volatilization [6]. Atrazine is degraded in three main ways in the environment [18]:
1) chemical hydrolysis of the Cl-C bond, producing hydroxyatrazine (non-phytotoxic); 2) N-dealkylation of carbon 4 and/or 6 producing deethylatrazine and deisopropylatrazine as well as diaminochloro-s-triazine, all of which are less phytotoxic than the parent compound; 3) microbial splitting of the triazine ring.

First-order chemical hydrolysis of atrazine to hydroxyatrazine has been reported as an important degradation pathway in soil [6]. The rate of hydrolysis is mainly controlled by soil pH and organic content. Adsorption to organic matter such as fulvic acids increases the hydrolysis of atrazine.

The DT50 of atrazine in soil is highly variable, ranging from 20 to 385 d or longer [15,18]. Half-lives of atrazine at pH 4 at 25°C, with and without 2% fulvic acid, were 1.7 d and 244 d respectively [15]. Atrazine was completely degraded by a mixed microbial culture, with or without the presence of other contaminant pesticides, although these did increase the time required for the degradation of atrazine by 2-3 fold [21]. Major microbial metabolites are deethyl and deisopropylated atrazine.

Losses due to runoff may reach 18% of the applied amount but tend to be <3% [18]. A loss of up to 15% of applied atrazine has been determined in Miami silt loam soil, 95.4% of which occurred in the dissolved phase [19]. When applied to 10 x 50 m plots at 0.88 kg ai/ha, 50% of atrazine disappeared from the surface layer in 35 d [20]. Peak concentrations were detected at a depth of 5-15 cm between d 8-45, reaching 52 µg/kg. Leachates were collected at a depth of 90 cm in a drainage system: the highest concentration of 65 µg/L was measured 1 month after treatment. The soil in this experiment was 29% clay, 49% silt and 22% sand, with 1% organic matter content and pH of 7.6 [20].

2.2.1.2 *Water and Sediment*

In southern Ontario, overall mean atrazine concentrations in Lake Erie tributaries during the late 1970s were up to 1.6 µg/L, with individual high values reaching 31.7 µg/L [24]. Bishop et al. [25] reported that atrazine was detected in the Holland River watershed during the 1990-92 growing season at 0.06-6.47 µg/L. In a study of herbicide runoff in paired agricultural watersheds of the Thames River in southern Ontario, Struger et al. [26] sampled extensively over a four-year period (1988-1991). Samples were collected during base flow periods (grab samples), high flow periods (8 samples over 12 h during storm events), and on a continuous (weekly composite flow-proportional samples). Atrazine concentrations in 7 of 129 (5.4%) grab samples exceeded the Canadian Water Quality Guideline for the protection of aquatic life of 1.8 µg/L [27]. Of 294 storm event sampling sessions, 146 (49.7%) samples exceeded the guideline. Of 185 weekly continuous samples, 13 (7%) exceeded this guideline. The maximum concentration was 95 µg/L.

In a survey of 8 Canadian tributaries of Lake Erie in 1998 and 1999, Struger et al. [28] detected atrazine in 8 of 127 samples. The maximum concentration was 4.9 µg/L. In a survey of 8 Canadian tributaries of Lake Ontario in 2000, Struger et al. [29] detected atrazine in 15 of 50 samples. The maximum concentration was 0.45 µg/L. In New Brunswick, a maximum concentration of 13.9 µg/L was detected in tile drainage of a treated field [30] while the maximum concentration in a receiving stream was 1.89 µg/L.

In the midwestern U.S. corn belt, the area of most intensive atrazine use, this chemical has been measured in tailwater pits draining irrigated fields at up to 250 μg/L [6]. The maximum sediment concentration of atrazine in these pits was 369 μg/kg. Shallow lentic waters in areas of heavy use have been found to contain residues of 2300 μg/L, with maximum concentrations as high as 49000 μg/kg in suspended sediments [22,23]. Surface water monitoring data from the midwestern states collected from 149 sites during 1991-1993 indicate that 52% of the sites exceeded the U.S. EPA's maximum contaminant level of 3 μg/L, with maximum residues of over 100 μg/L [5].

Degradation rates in water are highly variable. The DT50 in water has been estimated to range from 3-90 d or more and in sediment the range was 15-35 d [7]. A 17% loss was detected after 128 d in river water from an estuarine watershed.

The triazines are often detected in groundwater, which has been one of the main reasons for the recent restrictions and reductions in their use. A survey of 91 wells in southern Ontario during 1984 showed atrazine concentrations of 0.1-74 μg/L [31]. Results from a groundwater survey (1993-1994) by the National Water Quality Assessment Program in the U.S. revealed that atrazine was the most frequently detected pesticide, occurring in 38% of the 1034 sites sampled. The second most common chemical found was deethylatrazine, a metabolite of atrazine, with a detection rate of 34.2%. In that survey, the U.S. maximal limit of 3 μg/L was exceeded in only one groundwater sample, [32]. In a 1995 study of Iowa's groundwater, atrazine was found in 40% of 106 municipal wells and deethylatrazine in 35%. The maximum concentration of atrazine was 2.13 μg/L [33].

2.2.1.3 *Air and Precipitation*

The vapour pressure of atrazine is relatively low (6.9×10^{-6} at 25°C), which has led in some cases to the assumption that aerial transport is negligible. However, the vapour pressure of atrazine more than doubles between the temperatures of 20-30 °C. The combination of high surface temperatures, high wind speed and application to foliage enhances loss of atrazine by volatilization. At 40°C, 10% was volatilized from soil, compared with 50% from plant surfaces. As much as an 18-32% loss has been determined in soil at 35°C after 48 h.

Atrazine has been detected in alpine lakes in Switzerland, originating from rainwater contamination (0.6 μg/L) [34]. There is disagreement on the importance of dry versus wet deposition of atrazine. A recent project, the Lake Michigan Mass Balance project, sponsored by the EPA, states that dry deposition was not considered because studies have shown that it represents a minor fraction of total atrazine deposition to the lake [35]. However, in the Netherlands, aerial deposition of atrazine has been monitored outside the area of use. The calculated emission of atrazine from corn was 0.178 kg ai/ha/yr, which is 23% of the amount applied [36]. It was concluded that NOECs of sensitive species of plants may be exceeded within 200 m of treated areas and that dry deposition seems to be more important than wet deposition in that area.

Triazine herbicides were detected in nearly 85% of air samples collected outside of Paris, France in 1992-93 [37], with atrazine being found in 75% of samples. The concentration range measured in air was 0.03-2.0 ng/m^{-3} and in rainwater, 5-380 ng/L. These levels are similar to those found in the Great Lakes region in the U.S., where aerial concentrations reached 5 ng/m^{-3} during May-June treatment periods [38].

A recent Canadian review [39] indicated that low-level widespread contamination of terrestrial and aquatic environments by atmospheric transport of atrazine occurs year round. Loss by volatilization from treated fields in Maryland accounted for 2.4% of the applied amount. The importance of atmospheric transport in some areas may be equal to that of runoff losses. In Norway, residues of atrazine were detected in rainwater four years after its removal from the market [39].

Atrazine residues in precipitation [40].

Ontario:	to 0.44 µg/L (1992)
Eastern U.S.:	to 16 µg/L
Iowa:	to 40 µg/L
Minnesota:	to 2 µg/L
Europe:	0.08-2.0 µg/L

At the Experimental Lakes Area (ELA) in northern Ontario, located at least 100 km from any farming activity and 1000 km from the U.S. corn belt, total aerial concentrations of atrazine ranged from 0.02-0.09 ng/m^3 [39]. Maximum concentrations occurred in mid June, correlating with high use periods in the Midwest. Also, residue concentrations in air did not correlate with local temperature changes, indicating long-range transport. Precipitation was found to be the most important route of entry of atrazine into surface waters at the ELA stations. There, rainwater containing 0.03-51 ng/L atrazine was collected between 1990-1995, whereas in an untreated area in southern Manitoba, concentrations of up to 12.5 ng/L had been measured [39]. Gas exchange and particulate deposition also contribute to atrazine deposition into surface water, particularly early in the field season [39]. For example, at the ELA in 1995, particulate deposition accounted for 235 ng/m^2, gas exchange for 421 ng/m^2 and precipitation for 2006 ng/m^2.

A source of off-season input of atrazine to lotic systems is through groundwater recharge [10]. Groundwater is contaminated during the summer months and acts as a reservoir of pollution since atrazine is very stable in aquifers. Groundwater seepage into surface waters in the fall can therefore contribute to the total atrazine load [10].

The Canadian Water Quality Guideline for the protection of aquatic life for atrazine is 1.8 µg/L [27]. This value was derived from a study by Pratt et al. [41], which determined an MATC of 17.9 µg/L for a mixed microbial community. The NOEC was 10 µg/L, based on functional responses. In the U.S., there is no guideline for aquatic effects, however, the U.S. EPA is currently working to establish both freshwater and marine criteria for this herbicide, although the review process may take several years. The U.S. drinking water guideline is 3 µg/L, and the Canadian drinking water guideline is 5 µg/L [42].

2.2.2 Cyanazine

Off-target movement of triazines is associated with the dissolved phase in runoff as opposed to movement with eroded soil. Muir and Baker [43] studied the loss of cyanazine after a post emergence application of 3.3 kg ai/ha to tile-drained corn fields. Drain depth was 1.3 m and the soil was a sandy loam. Cyanazine was found in tile water at 0.01-0.68 µg/L, with concentrations reaching 1.06 µg/L in the second year of the study following a further application. The metabolite, cyanazine amide, was found at similar concentrations. The mean tile water concentration was 0.5 µg/L. Further studies confirmed that loss of cyanazine through tile drainage was less than 0.2% of the amount applied and generally less than 2% is lost in runoff [16].

Funari and co-workers [20] determined a 50% dissipation time of 28 d for cyanazine in the top layer of soil. In their study, cyanazine leached into tile drainage water (0.9 m deep) when applied at 0.9 kg ai/ha. The peak concentration in tile water reached 128 µg/L 1 month after application, the highest residue level of all six pesticides tested. The soil at the site contained 29% clay, 49% silt and 22% sand with 1% organic matter. The total amount of cyanazine recovered from leachate represented 0.1% of the applied amount.

In a similar study by Frank et al. [44] on clay loam soil in Ontario, the disappearance time of cyanazine was 181 d in 1987 and 90 d in 1988. The DT50 in soil was 12-27 d. Tile drainage samples indicated that little of the chemical leached through the soil after application at 2.4-2.6 kg ai/ha. In Ontario and Quebec, 85% of the applied herbicide was lost within 5 months in soil [45]. In Pennsylvania, cyanazine was detected in groundwater at 0.1-1.0 µg/L [46]. Application at 2.2 kg ai/ha resulted in well water concentrations of 3.4-3.6 µg/L. As much as 180 μg/L cyanazine was found in runoff solution, and 2300 μg/L in sediment 10 d post-application [47]. In the laboratory, the half-life of cyanazine in water was 14 d and in sediment it was 28 d [51].

Surface water measurements in southwestern Ontario in the early- to mid-1980s revealed that cyanazine concentrations were highest in the Thames River [48]. Where detectable, mean concentrations were about 2.6 μg/L, although only 45 of 440 samples had detectable residues. In surveys of tributaries of Lakes Ontario and Erie in 1998-2000, cyanazine was not detected in 177 samples [28,29]. During 1986, cyanazine was found in 34 of 422 surface water samples at municipal water works in Ontario [49]. Concentrations ranged between 0.08-6.8 μg/L.

In two Chesapeake Bay tributaries, cyanazine was detected at an annual mean of 31 ng/L, compared with 245 ng/L of atrazine and 121 ng/L simazine [50]. In a U.S. national groundwater survey, cyanazine was detected in only 1.8% of the sites sampled compared to 38% for atrazine and 18% for simazine [32].

2.2.3 Simazine

Simazine enters surface waters through similar input pathways as do the other triazines, although an additional source results from its use as an aquatic herbicide in farm ditches. When applied to corn on loam soil at 2.2 kg ai/ha, the maximum concentration of simazine was between 210-456 μg/L in runoff, although the field slope was 6-7% [52]. Concentrations decreased to 0.6-4 μg/L by 18 weeks post application.

About 0.3% of applied simazine was lost to the Wye River through runoff, with peaks of 300 μg/L, although maximum annual losses of 5.4% have been recorded [53].

The time until 50% disappearance in soil was found to be 28 d by Funari et al. [20] and the maximum concentration in tile water reached 58 μg/L within 30 d of treatment. In this particular study, high concentrations of metabolites were detected within a short period of time. Deisopropylatrazine and deethylatrazine, both common metabolites of triazine herbicides, were measured at up to 134 and 13 μg/L, respectively, in leachate.

In another extensive field study by Cogger et al. [54], simazine was found to be highly persistent in soil, having a DT50 of 128-424 d. Field treatments were of 1.1-4.5 kg ai/ha on alluvial soil. Simazine persisted from one year to the next, but did not leach much below 30 cm. The increased persistence was attributed to fall application and high volume of use. In well water samples, residues of 3.2-17 μg/L were detected, and did not decline significantly until 1 year after the last treatment. Non-equilibrium sorption was thought to enhance downward movement in the soil profile.

Dissipation in water is highly variable, with half lives ranging between 30-700 d [17]. In southern Ontario surface water samples collected between 1981-1985, simazine was seldom detected. Of the 202 samples collected at river mouths, only 8 contained simazine at a mean concentration of 1.1 μg/L [48]. It was detected in only 10 of 211 pond samples in southern Ontario between 1971 and 1985 with a range of 0.1-3 μg/L. Simazine was not detected in any of the surface water samples collected in Ontario between 1989 and 1994 in which atrazine residues were seen [26]. Similar results were obtained between 1998 and 2000. In a survey of 8 Canadian tributaries of Lake Erie [28], simazine was not detected in 127 samples. In a survey of 8 Canadian tributaries of Lake Ontario, Struger et al. [29] did not detect simazine in 50 samples.

In a national U.S. groundwater survey simazine was the third most frequently detected pesticide, and was found at 18% of the sites, with maximal concentrations of 1.3 μg/L [32]. Simazine has been detected in rain water in the central U.S. at 0.1-0.5 μg/L and in fog at up to 1.2 μg/L [17]. Simazine was detected at a mean concentration of 18-121 μg/L in some Chesapeake Bay tributaries [50] while in others it was found at up to 2.7 μg/L [55].

2.3 BIOCONCENTRATION AND METABOLISM

2.3.1 Atrazine

2.3.1.1 *Plants*

The metabolism of atrazine in plants has been recently reviewed [56]. Triazines are metabolized by three competing pathways: 1) Hydrolytic dehalogenation; 2) N-dealkylation and 3) glutathione conjugation. Metabolites produced by one pathway may undergo further metabolism by another pathway. Twenty eight atrazine metabolites have been identified in plants so far [56]. In atrazine-tolerant plants such as corn, atrazine is readily transformed to hydroxyatrazine which further degrades via dealkylation and hydrolysis of the amino groups [15]. These metabolites are generally less phytotoxic than the parent compound. Marshgrass translocated atrazine from the roots to the shoots within two d and produced metabolites such as 2-chloro-4-amino-6-ethylamino-s-triazine. In sorghum leaf sections, glutathione conjugates were detected.

N-dealkylation occurs in most plants and is the primary route of metabolism in species that lack the other pathways. The glutathione conjugation reaction is common in highly tolerant species such as corn and sugarcane. Intolerant species lack the ability to readily metabolize atrazine.

In the case of phytoplankton, adsorption and uptake from water is rapid [18,57] and a bioconcentration factor of 10-50 has been observed in green algae. The concentration period in green algae is 2-4 h prior to saturation and it has been shown that uptake of atrazine is strongly influenced by growth rate, whereas lipid content of cells plays no significant role.

Tang et al. [57] studied atrazine uptake in 8 species of freshwater algae and diatoms. Their studies indicate that 90% of uptake occurred in the first hour of exposure. Bioconcentration was species dependent, although accumulation of atrazine in green algae (5.5-13 µg/g) was consistently higher than in diatoms (0.3-1.7 µg/g). Algae concentrated atrazine to much higher levels than that of the medium. Green algae bioconcentrated 4-10 times more atrazine than diatoms and there was a good inverse correlation between cell volume and rate of bioconcentration. In that study, bioconcentration factors ranged from 8-200 [57].

2.3.1.2 *Animals*

In vitro rat liver studies determined that dealkylation of atrazine predominates over conjugation [2]. No evidence for dechlorination of chloro-s-triazines was observed and the major metabolites in the rat appear to be mono/di-N-dealkylated products. Eighty percent of a radiolabeled dose was eliminated in 72 h. *In vivo* studies with chickens found that deethylated products are more common and that the chlorine atom is replaced by a hydroxyl group. The highest concentration of atrazine was found in abdominal fat of chickens, but metabolites such as deethylhydroxyatrazine were highest in the liver.

Invertebrates such as cladocera and leeches concentrate atrazine to a lesser extent than algae [18], with bioconcentration factors of 2-50. A survey conducted in several Chesapeake Bay tributaries determined that although triazines were present in the water at the 0.1-0.4 µg/L range, oysters had no detectable residues [50]. A bioconcentration factor (BCF) of 11 was determined for the amphipod *Gammarus* exposed to atrazine for 3 d, whereas daphnids showed BCFs of 4.4 when exposed to 0.01-0.08 mg/L atrazine.

Uptake of ^{14}C atrazine in carp, from a solution of 1 mg/L, was found to occur at a rate of 0.16 mg/g/hr over 72 h. Concentrations of atrazine in the blood, gills and muscle reflected external levels, however, higher concentrations were observed in liver, kidney and intestines [6]. Bioconcentration was not found in brook trout, fathead minnows or bluegill sunfish exposed to 0.74 mg/L, 0.21 mg/L and 0.094 mg/L respectively for up to 44 weeks.

The metabolism of atrazine in animals was recently reviewed by Wu et al. [58]. N-dealkylation of the side chains is a major biotransformation process. Conjugation with glutathione was the next most important process leading to the formation of cysteine conjugates, sulfides and sulfoxides. In fish, an equilibrium is reached between atrazine uptake and elimination within 4-6 h. White fish and carp showed BCFs of 2-8 in water with 100 µg/L atrazine over a period of 4 months [18].

Zebrafish (for Latin names of vertebrate species see Appendix 3) of various stages of development were exposed to 135-500 µg/L atrazine for 48 h [59]. Results indicated that uptake saturation occurred within 5 h, and within 24 h, 70-85% of the herbicide was eliminated. However, only 4% of the compound was metabolized in juvenile fish. A BCF of <10 was determined.

2.3.2 Cyanazine

Cyanazine does not appear to bioaccumulate to a large extent in aquatic organisms. In microcosm studies by Yu et al. [60] cyanazine and its metabolites did not bioaccumulate after 35 d. No residues were found in fish caught in rivers having a mean concentration of 0.09 µg/L. Studies of microcosms treated with 1.12 kg ai/ha cyanazine revealed that macrophytes accumulated about 0.62 µg/g, while a crab accumulated 0.17 µg/g of N-deethylated cyanazine after 33 d [61]. None of the other test organisms, including green algae, mosquito fish and snails, had any detectable residues. The bioconcentration factor for macrophytes was 193.5 and for clams it was 16.1.

Cyanazine is rapidly metabolized in rats and dogs and is eliminated from the body within 4 d [2]. Dechlorination and glutathione conjugation have been documented. In plants, the metabolic degradation of cyanazine involves the removal of the ethyl group, hydration of the cyano group and exchange of the Cl atom with a hydroxyl group [16].

2.3.3 Simazine

Simazine does not bioaccumulate in the aquatic environment [17]. Most bioconcentration factors are less than 100 and simazine is rapidly metabolized in fish. No residues were found in brown bullheads, gizzard shad and black crappie in the Hillman Creek watershed in Ontario during 1974, where concentrations in the water were 0.1-3.6 µg/L [62].

2.4 TOXIC MECHANISM OF ACTION

The s-triazines are photosynthesis inhibitors. Photosynthesis inhibitors compete with plastoquinone II at its binding site and block the transport of electrons from photosystem II which gets electrons from the splitting of water molecules, thereby releasing oxygen. Blockage by inhibitors leads to the halting of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) production. Atrazine is a potent inhibitor of the Hill reaction and associated photophosphorylation [6]. The blockage of electron transport leads to chlorophyll destruction, inhibition of carbohydrate synthesis, reduction in the carbon pool and build up of CO_2 in the cell [7].

Plant death most commonly results from subsequent lipid peroxidation caused by triplet state chlorophyll and singlet oxygen, common products resulting from the inability to reoxidize plastoquinone [2]. In the absence of light, atrazine does not affect plants. The binding of the herbicide to the plastoquinone site is reversible, as photosynthesis increases in plants when transferred to clean water [7].

The inhibition of photosynthesis over longer periods of time reduces plant energy stores and results in plant death. Processes that are indirectly dependent on photosynthesis are also affected, such as transpiration, ion transport, opening of stomata and others [18]. Atrazine can upset the phytohormone and ion balance, which may lead to substantial disruptions of overall metabolism, affecting enzymes and protein synthesis.

2.5 ACUTE TOXICITY

2.5.1 Atrazine

2.5.1.1 *Phytoplankton*

Many studies have evaluated the effects of atrazine on algae, revealing a wide range of sensitivity among species, having EC50 values range from 4 to >1000 µg/L [63-68,95-97]. Concentrations as low as 1 µg/L can cause inhibition in some algae [63,64]. The maximum recommended safe level of atrazine to diatoms is 10 µg/L [64] although temporary inhibition of chlorophyll production has been reported in the range of 1-5 µg/L. Phytoplankton are the most acutely sensitive group of organisms to the triazines followed by aquatic macrophytes, benthos, zooplankton and fish [7,63,65,66].

In a recent study [66] with four species of green algae and four species of diatoms, atrazine at 10 µg/L caused some inhibition of chlorophyll-a production. This was a slightly more sensitive endpoint than cell density. However, in some species this concentration caused an increase in these parameters. Growth inhibition became most evident at 100 µg/L for green algae and at 250 µg/L for most diatoms. In this study, the most sensitive group were the *Chlamydomonas* spp. with a 7-d EC50 value of 46.5 µg/L, based on chlorophyll-a content. In another study, *Selenastrum capriconutum*, consistently one of the most sensitive green algae to the triazines, showed a 96-h EC50 of 4 µg/L, and an NOEC of 0.5 µg/L, based on cell count [67].

The effects of 23 pesticides were investigated on 10 species of algae and the macrophyte *Lemna minor* [68]. Atrazine inhibited ^{14}C uptake after 24 h of exposure by 84-99% in 9 of the 10 algae at a concentration of 2667 µg/L. This level was determined to be Expected Environmental Concentration (EEC) following a direct overspray in shallow water (15cm) [68].

In a New Brunswick field survey [69] a 2-3 fold reduction was noted in the number of algal species and cell counts in a stream receiving tile drainage containing atrazine at a maximum concentration of 13.9 µg/L. The concentration in the stream reached 1.89 µg/L and the effect was evident only within 60 m downstream of the tile drain.

The toxicity of atrazine transformation products has been found to be lower than that of atrazine [6,7,18]. The parent compound is 7-10 times more toxic to algae than the most potent transformation products deethylated and deisopropylated atrazine [67].

2.5.1.2 *Aquatic macrophytes*

Few studies have been conducted on the short term effects of atrazine on macrophytes, although the available data indicate that duckweed (*Lemna gibba*) is one of the most sensitive vascular plants, and water milfoil (*Myriophyllum spicatum*) is one of the most resistant (EC50s of 174 µg/L and 3700 µg/L, respectively) [6,7]. Atrazine is quickly translocated to the shoots even at concentrations as low as 20 µg/L. Significant inhibition of photosynthesis occurs at 77 µg/L in some plants.

Non-lethal inhibition of photosynthesis is often reversible once the herbicide is removed. A concentration of 650 µg/L reduced photosynthesis by 85% within 4 h in pondweed (*Potamogeton perfoliatus*) [70]. Horned pondweed (*Zannichellia palustris*) exhibited a 50% reduction in photosynthesis at 91 µg/L within 2 h [71]. In wigeon grass (*Rupia* sp.), a 1% inhibition was seen at 20 µg/L.

Irreversible effects in macrophytes are generally not seen over short time intervals at environmental concentrations (<30 µg/L). Such effects occur at concentrations of 320-1200 µg/L with exposure times of 2-4 weeks [18].

2.5.1.3 *Invertebrates*

The acute toxicity of atrazine is moderate, although highly variable, among invertebrates. Indirect effects, however, have been documented at levels as low as 20 µg/L. These effects will be discussed in more detail under chronic toxicity.

Significant differences were noted in the pattern of emerging adult insects with more individuals emerging earlier in microcosms treated with 5 µg/L atrazine [71]. Similar effects were observed by Dewey [72] who recorded earlier emergence of herbivorous insects from artificial ponds treated at 20 µg/L. One possibility is that a reduction or modification of the algal food supply triggers earlier emergence; an alternative explanation may be that atrazine caused a direct physiological response in some insects resulting in early emergence.

Most invertebrates are quite insensitive to atrazine, having acute EC and LC50 values of 300-4000 µg/L [6,7,18,65]. The most sensitive invertebrate tested was the saltwater calanoid copepod *Acartia tonsa* having a 96-h LC50 of 94 µg/L [73]. The egg hatchability of caddisfly seems to be quite sensitive to atrazine, having an LC50 of 22 µg/L [74]. Midge larvae had a 48-h LC50 of 720 µg/L [75] whereas the amphipod *Gammarus* showed mortality at 2400 µg/L and an LC50 of 5700 µg/L.

Atrazine was found to increase the toxicity of 4 of 5 organophosphorus (OP) insecticides to a chironomid midge in a synergistic manner [76]. Methyl parathion, trichlorphon, chlorpyrifos and malathion were more than additively toxic in binary mixtures with atrazine to *Chironomus tentans* in 96-h laboratory exposures. A less additive response was observed when a mixture of atrazine and methoxychlor was used, however, a ternary mixture of atrazine + methoxychlor + methyl parathion was also synergistic. The highest concentration of atrazine alone was non-toxic.

Atrazine reduced the toxicity of the OP, mevinphos [76]. The authors stated that a likely mechanism may involve the induction of cytochrome-p450 Mixed Function Oxidases (MFO) by atrazine, which has been documented in insects [77]. Many OP insecticides, but not mevinphos, are activated to their more potent oxon analogues by MFO enzymes; mevinphos however, as a member of the phosphate class of OPs, is instead deactivated by MFOs [76].

2.5.1.4 *Fish and Amphibians*

Mortality in fish and amphibian species only occurs at high concentrations, in the range of 220-10000 µg/L [6,65] which are well above expected environmental concentrations in almost all places. Among the most sensitive fish are rainbow trout, having a 96-h LC50 of 4500 µg/L. Early life stages of fish are more sensitive: for example, channel catfish exposed from the fertilized egg through 96 h post-hatch has an LC50 of 220 µg/L [78]. Similarly, brook trout fry showed mortality at 240 µg/L, while adults showed no signs of toxicity at 720 µg/L [75]. Rainbow trout embryos are almost an order of magnitude more sensitive to atrazine than adults with an LC50 of 870 µg/L [79].

Although lethal effects are not expected in the environment for vertebrate species, a number of studies have shown that sublethal physiological responses in some fish are indicative of stress from exposure to atrazine. In a recent experiment, conducted with goldfish, a number of intriguing behavioural responses were observed at very low, environmentally relevant concentrations [80]. Following a 24-h exposure period, a significantly higher occurrence of burst swimming was noted at 0.5 µg/L, the lowest test concentration. Fish grouping was reduced at 5 µg/L and the number of surfacings was significantly higher in the exposed group. Grouping and sheltering was also reduced in response to a skin extract solution (response to an alarm pheromone) when exposed to 5 µg/L atrazine. Such behavioral alterations could cause reduced predator avoidance responses and reductions in survival in wild species of fish exposed to low levels of atrazine.

A significant depression in plasma protein was found in rainbow trout exposed to 50 µg/L atrazine for 10 d, while plasma glucose was significantly higher than control at 430 µg/L [81]. The latter concentration reduced growth rates. Two other species of riverine fish tested in that experiment (*Galaxias maculatus* and *Pseudaphritis arvilii*) demonstrated even higher sensitivity, including altered blood composition (altered leucocrit) at 3-50 µg/L, depressed muscle DNA levels at 0.9-10 µg/L, and depressed GSH levels above 3 µg/L. Atrazine at 10 µg/L caused significant alterations in blood glucose levels within 6-12 h of exposure in carp.

Anuran species are of similar sensitivity as fish to atrazine. The late larval stage is generally the most susceptible stage in frogs. The LC50 for bullfrogs (exposed from pre-hatch to 4 d post-hatch) was 410 µg/L [78] a concentration which produced deformities in 3% of hatchlings. The corresponding value for leopard frogs was 7680 µg/L. Howe et al. [82] detected synergistic effects of a 50:50 mixture of atrazine and alachlor in both leopard frogs and American toads. The 96-h LC50 of atrazine alone was 14500 µg/L and 10700 µg/L, to leopard frogs and toads, respectively, while these values for the mixture were 2100 and 1500 µg/L, respectively.

2.5.2 Cyanazine

The database on the ecotoxicological effects, or even standard toxicity tests with cyanazine is very limited [16]. In the only recent study found in the open literature, cyanazine was determined to be about 9-fold more toxic to the green alga *Selenastrum capricornutum* than atrazine (96-h EC50s of 27 µg/L versus 235 µg/L) [83]. The NOEC and LOEC for inhibition of chlorophyll-a production were determined to be 9 µg/L and 19 µg/L, respectively. The macrophyte *Lemna minor* was less sensitive to cyanazine than to atrazine (EC50s or 705 µg/L versus 153 µg/L). In some algae, 52 µg/L cyanazine stimulated chlorophyll-a production while 416 µg/L reduced it by 40% [84]. *Chlorella* was inhibited by 38% at 208 µg/L [84].

Cyanazine is moderately toxic to fish and invertebrates. The most sensitive organism tested was the amphipod *Gammarus fasciatus*, having a 96-h LC50 of 2000 µg/L [85]. The lowest reported LC50 for a fish was 4800 µg/L [86]. Rainbow trout, fathead minnows and cladocera have LC50 values of 9000 µg/L, 16000 µg/L, and 84000 µg/L, respectively [87, 88].

The sublethal effects of cyanazine were investigated in a 10-d study [81]. Rainbow trout showed elevated GST activity at 80 µg/L and above. In a crustacean (*Paratya australiensis*), interestingly, up to a 57% inhibition of acetylcholinesterase (AChE) activity was seen in tail muscle at a concentration of 80 µg/L and higher. The Canadian Water Quality Guideline for the protection of aquatic life from the adverse effects of cyanazine is 2.0 µg/L [16,27]. That guideline, however, established in 1991, did not consider the above mentioned studies on *Selenastrum* as they were not available at the time. The guideline is based on a study with *Chlorella* (EC50= 208 µg/L) and a safety factor of 100 due to lack of sufficient data [16]. This guideline was set as an interim guideline, and was meant to be updated as new data become available.

2.5.3 Simazine

The database on the ecotoxicological effects, or even standard toxicity tests with simazine is very limited [16]. Periphyton were unaffected at a concentration of 100 µg/L simazine in microcosm studies, but were inhibited by 95% by a concentration of 1000 µg/L. Recovery of the periphyton community began 1 week after treatment [89]. Algal community LC50s were found to range between 100-1000 µg/L, although a community structural change was noted at 50 µg/L or higher, which included reduced diversity and altered dominant species composition [90]. In the green algae, *Selenastrum capricornutum,* 1240 µg/L simazine inhibited chlorophyll-a production by 50% in a 96-h exposure [83]; this value for the macrophyte *Lemna* was 166 µg/L.

Simazine toxicity to invertebrates is low. Amphipods, glass shrimp and crayfish were not affected at 100 µg/L and the 48-h EC50 for *Daphnia* and seed shrimp was 1000-3200 µg/L, respectively [92]. Benthic invertebrates are even less sensitive, having LC50 values of up to 28000 µg/L. Stonefly and mayfly larvae have LC50s of 1900-5000 µg/L [93].

The acute toxicity of simazine to fish and amphibians is also low [17]. No mortality was seen in coho salmon smolts at 2500 µg/L, but pumpkinseed died at 2000 µg/L. Striped bass had an LC50 of 250 µg/L at 96 h [94]. At this concentration of simazine, an excessive kill of vegetation and algae can lead to oxygen depletion and indirect stress to fish.

Table 2.3 Acute aquatic toxicity data for the triazine herbicide atrazine. Vertebrate and invertebrate species indigenous to Ontario are identified by an (I) after the species name.

ATRAZINE

Species	Common name	Chemical formulation	Dose method	Test time	LC50/EC50(µg/L)	Reference
Algae						
Cyclotella gamma	Diatom	Technical 99.8%	Static	7-d	494	66
Cyclotella meneghiniana	Diatom	Technical 99.8%	Static	7-d	959.4	66
Synedra acus	Diatom	Technical 99.8%	Static	7-d	259	66
Synedra radians	Diatom	Technical 99.8%	Static	7-d	337	66
Chlamydomonas sp.	Green alga	Technical 99.8%	Static	7-d	46.5	66
Chlorella sp.	Green alga	Technical 99.8%	Static	7-d	72.9	66
Pediastrum sp.	Green alga	Technical 99.8%	Static	7-d	536	66
Chlamydomonas sp.	Green alga	Technical 99.8%	Static	7-d	80.6	66
Selenastrum capricornutum	Green alga	-[a]	Static	96-h	147	95
Dunaliella tertiolecta	Green alga	-	Static	96-h	132	95
Selenastrum carpicornutum	Green alga	Technical	Static	96-h	117	96

Table 2.3 Continued

Species	**Common name**	**Chemical formulation**	**Dose method**	**Test time**	**LC50/EC50(μ g/L)**	**Reference**
Chlorella vulgaris	Green alga	Technical	Static	96-h	94	96
Chlamidomonas reinhardi	Green alga	Technical	Static	96-h	176	96
Selenastrum carpicornutum	Green alga	-	Static	72-h	283	97
Anabaena flos-aquae	Green alga	-	Static	72-h	58	97
Microcystis sp.	Blue green alga	Technical	Static	96-h	90	96
Macrophytes						
Myriophyllum sp.	Water milfoil	Atrazine 4L	Static	5-d	20000	98
Lemna sp.	Duckweed	Technical	Static	96-h	92	96
Potamogeton perfoliatus	Pondweed	-	Static	4-h	80	70
Rupia maritima	Wigeon grass	-	Static	2-h	102	71

Table 2.3 Continued

Species	**Common name**	**Chemical formulation**	**Dose method**	**Test time**	**LC50/EC50(µg/L)**	**Reference**
Invertebrates						
Acartia tonsa (I)	Copepod	-	Static	96-h	94	73
Daphnia magna (I)	Water flea	-	Static	48-h	3600	99
Daphnia magna (I)	Water flea	-	Static	48-h	6900	75
Daphnia pulex (I)	Water flea	-	Static	18-h	600	76
Hyallella azteca (I)	Scud	-	Static	48-h	8800	76
Gammarus fasciatus (I)	Scud	-	Static	48-h	5700	75
Mysidopsis bahia	Shrimp	-	Flow through	96-h	1000	73
Chironomus riparius (I)	Midge	-	Static	48-h	1000	99
Chironomus tentans (I)	Midge	-	Static	48-h	720	75

Table 2.3 Continued

Species	Common name	Chemical formulation	Dose method	Test time	LC50/EC50(μg/L)	Reference
Fish and Amphibians						
Lepomis macrochirus (I)	Bluegill sunfish	-	Flow through	96-h	>8000	75
Oncorhynchus mykiss (I)	Rainbow trout	Technical	Static	96-h	4500	100
Salvelinus fontinalis (I)	Brook trout	-	Flow through	96-h	6300	75
Pimephales promelas (I)	Fathead minnow (fry)	-	Static	96-h	520	75
Pimephales promelas (I)	Fathead minnow (adult)	-	Static	96-h	15000	75
Ictalurus melas	Catfish	80% WP	Static	96-h	35000	100
Carassius carassius	Crucian carp	80% WP	Static	96-h	10000	100
Cyprinus carpio	Carp	94% Technical	Static	96-h	188000	101
Rana pipiens (I)	Leopard frog	-	Flow through	96-h	7680	78
Rana catesbeiana (I)	Bullfrog	-	-	96-h	410	78

[a] information not provided

Table 2.4 Acute aquatic toxicity data for the triazine herbicide cyanazine. Vertebrate and invertebrate species indigenous to Ontario are identified by an (I) after the species.

CYANAZINE

Species	Common name	Chemical formulation	Dose method	Test time	LC50/EC50(μg/L)	Reference
Daphnia magna (I)	Water flea	-[a]	Static	48-h	84000	88
Oncorhynchus mykiss (I)	Rainbow trout	80 WP	Static	96-h	9000	87
Pimephales promelas (I)	Fathead minnow	-	Static	96-h	16300	16
Labeo rohita	Carp	-	Static	96-h	4800	86
Selenastrum carpicornutum	Green alga	-	Static	96-h	27	83
Lemna minor	Duckweed	-	Static	96-h	705	83

[a] information not provided

Table 2.5 Acute aquatic toxicity data for the triazine herbicide, simazine. Vertebrate and invertebrate species indigenous to Ontario are identified by an (I) after the species.

SIMAZINE

Species	**Common name**	**Chemical formulation**	**Dose method**	**Test time**	**LC50/EC50(µg/L)**	**Reference**
Daphnia pulex (I)	Water flea	80% commercial	Static	48-h	5,300-92,000	102
Daphnia magna (I)	Water flea	Technical	Static	48-h	1000	92
Tendipedidae sp (I)	Midge	80% commercial	Static	96-h	28,000	103
Pimephales promelas (I)	Fathead minnow	80% commercial	Static	96-h	510,000	87
Pimephales promelas (I)	Fathead minnow	4% granular	Static	96-h	5,000	87
Cirrhina mrigala	Carp	-[a]	Static	96-h	7,500	104
Bufo bufo	Common toad	-	Static	48-h	>100,000	6
Scenedesmus quadricaude	Green alga	80% commercial	Static	-	200,000	105
Selenastrum carpicornutum	Green alga	Technical	Static	-	1240	83
Selenastrum carpicornutum	Green alga	Princep 4G	-	-	2.24 (oxygen evolution)	106
Lemna minor	Duckweed	Technical	Static	96-h	166	83

[a] information not provided

2.6 CHRONIC TOXICITY AND ECOLOGICAL EFFECTS

2.6.1 Phytoplankton

In the Platt River in Nebraska, average concentrations of atrazine at base flow are around 1 µg/L [64]. At this concentration, atrazine significantly affected the growth rate of a benthic diatom, although only during day 1 of a 67-d exposure period. When the same algae were subsequently exposed to higher concentrations of atrazine (83-3500 µg/L) for up to 12 d, a reduced tolerance was noted in diatoms. The effects were manifested in declined growth at 7 d of exposure to 83 µg/L atrazine [64].

Bester et al. [107] tested the effects of atrazine on four species of marine diatoms in a 15-d mesocosm study using concentrations typically found in the North Sea (0.12 µg/L), the coastal waters of Germany (0.56 µg/L) and at the mouth of the river Elbe (5.8 µg/L). Although no replicates were used, a distinctive increase in the dissolved organic nitrogen and phosphorus (DON and DNP) was noted at all treatment levels and showed a dose dependent response. This effect was attributed to forced excretion and/or lysis of cells, a theory which was supported by higher amino acid concentrations in solutions with higher atrazine levels and lower chlorophyll-a contents [107]. Maximum primary production was lower in the mesocosms spiked with 0.56 µg/L atrazine than in control mesocosms. The authors concluded that atrazine concentrations above 0.12 µg/L may change the function of phytoplankton communities and exert selective pressure. Similar responses were also documented by Kasai and Hatakeyama [108].

In a multi-species stream mesocosm experiment, conducted at the U.S. EPA's Monticello Ecological Research Station, Detenbeck et al. [10] investigated the response of periphyton, macrophytes, invertebrate and fish populations to atrazine. Treatment consisted of a stepped exposure regime of four increasing concentrations: 15 µg/L, mid-May to early June, 25 µg/L until mid-July, 50 µg/L until mid-August and 75 µg/L until early September. Periphyton gross productivity was significantly reduced at the lowest concentration tested (15 µg/L). In general, exposure to atrazine tended to shift periphyton community composition from dominance by filamentous green and blue green algae to smaller more tolerant diatoms and chrysophytes [10]. Maximum dissolved oxygen levels were significantly reduced at 15 µg/L atrazine compared to controls (5.6 mg/L versus 7.3 mg/L), while nutrient content in water was significantly higher at ≥25 µg/L, including NH^{4+}, DOP and DON. These changes were deemed to be related to reduced periphyton productivity.

The lowest previously determined effect level for atrazine was 24 µg/L for periphyton [109]. In that study, a temperature interaction was found: periphyton were significantly affected at 25 °C but not at 10 °C. Respiration was significantly reduced at 25 µg/L and stimulated at 75 µg/L. Chlorophyll-a content was not affected by any of the treatments. Development of periphyton resistance and/or a shift in community composition was noted at ≥50 µg/L atrazine. Other investigations have revealed selection for atrazine resistant strains of *Chlamydomonas* sp. and *Navicula* sp. [110].

Many studies indicate that algae frequently recovers from initial impacts of atrazine exposure. Green algal colonies showed reduced chlorophyll levels at 20 µg/L atrazine after 7 d of exposure in outdoor microcosms [111], but reductions were no longer apparent by day 14. This finding supports that of deNoyelles et al. [112] who found that 20 µg/L atrazine had a significant negative impact on algal productivity and biomass in mesocosms, which was followed by recovery after 7 d [111]. Even at 153 µg/L atrazine a similar recovery was noted in algal cell density and primary productivity by 14 d [111]. The study of Larsen et al. [113] revealed that ^{14}C uptake in algal species recovered after 10 d from exposure to 60 and 100 µg/L atrazine, following an immediate decrease upon treatment. Chlorophyll-a production in algae was stimulated between 60-200 µg/L, while significant inhibition only occurred at 1000 µg/L. The increase was thought to be due to an adaptive compensatory mechanism similar to that observed in aquatic plants receiving low light [113].

In outdoor mesocosm tests in Kansas, primary productivity as measured by ^{14}C uptake was not clearly affected at 20 µg/L, but was reduced at 100 and 200 µg/L for two weeks [113]. It returned to control levels after two weeks and then decreased again after another two weeks, remaining depressed for 4 months. After that time, treatment and control mesocosms were again similar for this endpoint. Chlorophyll content was not affected at 20 µg/L, but was reduced at 100 µg/L; the EC50 was 82 µg/L [113]. Similar effects on algae were observed by Fairchild et al. [114] in a 0.1 ha mesocosm treated with 50 µg/L atrazine. Phytoplankton chlorophyll-a content increased slightly 1 week post treatment and thereafter decreased below control levels.

Pratt et al. [41] used mixed microbial communities, comprised of 150-200 species of bacteria, protozoa, algae, fungi and metazoans, to study the effects of atrazine on structural and functional endpoints. Protozoan species numbers were decreased after a 21-d exposure to 337 µg/L, while substrate colonization rate was significantly reduced at a concentration of only 3.2 µg/L. Primary productivity of the colonies was depressed at ≥10 µg/L throughout the test and on day 21 was significantly lower at ≥32 µg/L atrazine. In fact, oxygen production and the ability of communities to sequester calcium and magnesium ions were the most sensitive measures of toxicity, having a Maximum Allowable Toxicant Concentration (MATC) of 17.9 µg/L [41]. Lower concentrations (3.2-32 µg/L) caused an increase in species numbers and increased chlorophyll-a content by as much as 57%; however, these functional endpoints showed no hormetic response at low concentrations and were more sensitive than structural endpoints.

The above results suggest that loss of sensitive species and resultant increase in growth of insensitive species might be responsible for the observation of increased chlorophyll at low concentrations. In other studies, photosynthetic function, as measured by ^{14}C uptake per chlorophyll content, has also been found to be significantly reduced by atrazine at low concentrations, even though algal biomass was increased [115]. Similarly, functional endpoints such as oxygen content and conductivity were the most sensitive community responses observed by Juttner et al. [9] during a 60-d field study. Dissolved oxygen content decreased by about 5% within 6 d after treatment at 10 and 22 µg/L atrazine, whereas declines of 28% and 65% were seen at 68 and 182 µg/L. By the end of the study, no difference was evident in the oxygen content of the control and 5 and 10 µg/L treatment levels. There was a 20% reduction in oxygen content at 22 µg/L. Biological structural responses such as cell density were not readily apparent below 182 µg/L for phytoplankton. Diatoms were the least sensitive group of algae to atrazine and dominated community composition at 182 µg/L and higher. This concentration prevented the seasonal bloom of other algae.

In a recent report by Berard and co-workers [116] the effects of ecologically relevant concentrations of atrazine were investigated on the seasonal periodicity of Lake Geneva phytoplankton communities over a period of 4 years. The hypothesis was that, as seasonal changes take place in phytoplankton community composition, the sensitivity of the community to herbicides also changes. During March and April, the community was dominated by small, fast growing diatoms and cryptophytes, whereas during the clear water phase (caused by zooplankton grazing) in June, chlorophytes and cryptophytes dominated. Exposure to 10 µg/L atrazine always acted to restructure the algal community by disturbing species composition. The greatest change in community composition was produced during the clear water phase and the least during the early spring bloom. This finding indicates seasonal variation in susceptibility of algae to atrazine. The clear water phase coincides with maximal atrazine concentrations during early summer and it is thought that any indirect effects on zooplankton are most likely to occur following exposure during this time period. *Chlorella vulgaris* and similar chlorophytes were drastically inhibited at 10 µg/L, whereas tolerant blue green species such as *Oscillatoria* and *Nitzchia* were stimulated. In laboratory monocultures, these tolerant organisms were not stimulated by these concentrations, indicating that it is the elimination of sensitive species of algae that is responsible for enhanced growth of tolerant species, due to reduced competition [116]. Therefore, the herbicide acts as an additional factor in determining community structure and thus can exert a selection pressure. The tolerant groups of phytoplankton are believed to be able to substitute autotrophic behaviour with heterotrophic behaviour.

2.6.2 Macrophytes

Although most macrophytes are less sensitive to triazine herbicides than algae, having EC50 values of 22-1000 µg/L, significant rates of mortality (50-100%) have been reported in aquatic vascular plants from exposure to as little as 12 µg/L [117]. Annual additions of atrazine during a 3-year study period, producing a final concentration of 20 µg/L, reduced macrophyte coverage in experimental ponds by about 90%; recovery was not observed, and a change in species composition was seen [6,118].

Exposure to atrazine for 14 d reduced the wet weight of the submergent macrophytes *Ceratophyllum, Najas, Elodea* and *Myriophyllum* sp. at EC50s of 22, 24, 21, and 132 µg/L, respectively [96]. The floating macrophyte, duckweed (*Lemna gibba*) had an EC50 for frond biomass of 50 µg/L [119]. Interspecific variability in sensitivity to atrazine was less than one order of magnitude, as was the case for another triazine (metribuzin) whereas alachlor and metolachlor exhibited a range of sensitivity spanning close to two orders of magnitude [96].

Correll and Wu [117] determined a 47-d LC50 of 12 µg/L for *Vallisneria americana* in estuarine microcosms, although the salinity of the test water may have affected the response of the plant [6]. In comparison, Forney and Davis [120] found that *Vallisneria* had an EC50 of 163 µg/L for growth in freshwater tests.

There was no evidence of a significant effect on submerged macrophytes or phytoplankton from exposure to a single treatment of atrazine at 10-100 µg/L in wetland microcosms [99]. Although aquatic gross primary productivity was reduced significantly (23%) at 10 µg/L, recovery occurred by day 7. Oxygen production was significantly reduced in one macrophyte and stimulated in another at 5 µg/L [121]. Growth of pondweed (*Potamogeton perfoliatus*) was reduced by 50% at 30 µg/L in estuarine microcosms. Tuber development in *Vallisneria americana* was reduced at 4 µg/L atrazine [122].

Dry weight of sage pondweed (*Potamogeton pectinatus*) was a more sensitive endpoint than wet weight or number of rhizome tips, during a 28-d partial life-cycle study in estuarine microcosms [123]. At a salinity of 1 ppt, the NOEC, LOEC and MATC were 15, 30 and 21.2 µg/L, respectively; at 12 ppt salinity the MATC was reduced to 5.3 µg/L.

A study by Detenbeck et al. [10] revealed that coontail (*Ceratophyllum demersum*) was more sensitive than cattail (*Typha*) or *Elodea* in a flow-though stream experiment. At a concentration of 50 µg/L, there was an increase in the stem length/weight ratio after 6 d, indicating a reduction in dry weight and or elongation of the stem. Previous studies have also shown stem elongation in *Potamogeton* and *Myriophyllum* from exposure to 5-50 µg/L atrazine [121].

deNoyelles et al. [112] demonstrated a shift in macrophyte community composition in mesocosms treated with 20 µg/L atrazine and higher from one dominated by *Potamogeton* and *Najas* to one dominated by the macroalga *Chara*. At 100 µg/L or higher, *Chara* was the only plant to survive in the ponds.

In mesocosms treated with 50 µg/L atrazine, *Naja* was nearly eliminated over a period of 4 months and was replaced by *Chara* as the dominant macrophyte [114]. Atrazine did not significantly alter total plant biomass or system primary productivity but did significantly reduce gross photosynthesis and respiration one week after treatment, so that the production to respiration ratio (P/R) did not change.

An altered macrophyte community composition, although not significantly affected in terms of biomass or photosynthesis, may nonetheless have significant adverse impacts on other communities such as zooplankters and fish that rely on them for food and shelter. This may especially be the case since the number of macrophyte species present at any particular habitat may be quite low i.e., 2-3 spp. If the number of plant species is reduced to one or two, it is conceivable that invertebrate succession during the season may be altered. Such indirect impacts on invertebrates have been found in multispecies experiments as will be described. The question of the ecological effects of reduced biodiversity is one that must be confronted in ecotoxicological studies.

2.6.3 Invertebrates

In a laboratory study, *Chironomus* midge larvae exposed through two generations to 230 µg/L atrazine showed reduced hatchability, increased mortality, retarded development and reduced rate of emergence [75]. The NOEC was determined to be 110 µg/L. *Daphnia magna* survival was unaffected by 1150 µg/L atrazine for three generations (21 d) but the number of young produced per female was significantly lower in the first generation [75]. Survival of the amphipod *Gammarus fasciatus* was reduced by 940 µg/L atrazine during 30 d of exposure but was not reduced at 490 µg/L, even after 119 d. However, reproductive toxicity impaired the survival of offspring at 140 µg/L [75].

Several invertebrate community studies have been conducted with atrazine in field situations using mesocosms or whole ponds. Kettle et al. [118] reported that zooplankton communities appeared to be altered in mesocosms treated with 20 µg/L atrazine, which was thought to be due to the impact on the phytoplankton community. Benthic invertebrate populations were also altered in the ponds, as well as the production of bluegill sunfish. Stomach content analysis of bluegills showed a reduced amount and diversity of zooplankters. Dewey [72] reported a significant reduction in emerging chironomids from mesocosms treated with 20 µg/L over 3 years. Benthic species richness, equitability and total emergence all declined significantly with atrazine treatment. Herbivorous insects underwent greater relative reductions than did predatory insects and the emergence periods of several species in that category were also altered.

The population density of cladocerans in ponds treated at 20 µg/L was lower than that in control ponds even one year after contamination [18]. The most sensitive effect concentration for invertebrates in outdoor enclosures was found to be 0.1 µg/L by Lampert et al. [124] in which herbivorous zooplankton were reduced in abundance.

In a stream mesocosm study [10] *Daphnia* bioassays conducted at 15 µg/L indicated significant effects, although no such effects were found at 25-75 µg/L atrazine. The authors speculated that since system wide productivity was low, a lack of food may have contributed to the observed response.

In a multi-species microcosm study, including communities of benthos, insects and algae, no significant impacts were observed on insect abundance from exposure to 5 µg/L atrazine over a period of 14 d [71]. There was greater variability in invertebrate abundance and an overall shift towards earlier insect emergence in treated microcosms, suggesting altered community dynamics. Indirect effects on zooplankton were also reported by Juttner et al. [9] during a 6 week mesocosm study. Total numbers of the cladoceran *Daphnia longispina* declined in all enclosure following treatment at 5-318 µg/L. This experiment included 3 treatments of atrazine and reported concentrations were the measured means over a period of two months. This was accompanied by reduced egg ratios between day 3 and 21. Some recovery was noted after day 21, but males had no ephippia at the highest treatment level. *Copepod nauplii* increased in low dose ponds (up to 22 µg/L); similarly, rotifer taxa declined in abundance in all enclosures within the first two weeks.

Van den Brink et al. [125] detected only slight reductions in primary productivity over 7 weeks in multi-species microcosms exposed to 5 µg/L atrazine, and observed no significant effects on cyclopoid and cladoceran species or on the amphipod *Gammarus* and the rotifer *Keratella*.

2.6.4 Fish and Amphibians

Survival of juvenile fathead minnows was not affected in stream mesocosms treated with 15-75 µg/L atrazine for 10-13 d [10]. Weight and length of fish were also unaffected. Caged leopard frog tadpoles were exposed for 41 d in treated streams. No significant effects were observed on percent transformation or hind leg length, although tadpole development tended to be accelerated in treated mesocosms [10]. On the other hand, DeNoyelles et al. [cited in 10] detected a significant difference in biomass of bullfrog tadpoles in the Kansas mesocosms at 20 µg/L atrazine at the end of the third season. This reduction was attributed to loss of macrophytes (90% decline in area coverage) as spawning area and refugia as well as loss of periphyton food resources. The lack of demonstrated effect on leopard frog tadpoles in the stream exposures may have been due to the fact that tadpoles were stocked into the wetlands and were protected from predation by cages. Also, bullfrogs are inherently more sensitive to atrazine than are leopard frogs (96-h LC50s of 410 versus 7680, respectively [78]).

Kettle et al. [118] also detected changes in food intake and reproductive behaviour in channel catfish, gizzard shad and bluegill sunfish after 136 d of exposure to 20 µg/L atrazine in mesocosms. Over 70% of the applied atrazine was still present in water at that time. The amount of ingested food in the fish were clearly lower in the treated mesocosms, indicating a limited invertebrate food supply, which led to increased cannibalism of juvenile fish.

Macek et al. [75] reported that growth and survival of brook trout fry was reduced at 240 µg/L during a 44 week exposure period, while adult mortality, egg production and hatchability was unaffected at 720 µg/L. The MATC for chronic effects was 60 µg/L in brook trout. Bluegill sunfish showed no adverse effects on survival, growth or hatching from an 18 month exposure to 95 µg/L atrazine; similarly, no effects were seen in fathead minnows after 43 weeks of exposure to 213 µg/L [75].

Sublethal physiological effects have been reported in several fish species at low concentrations of atrazine e.g. 5-40 µg/L [18,79,126,127]. Such responses include kidney and liver damage and metabolic and hormonal alterations. Hepatic perturbations were seen in juvenile grey mullet exposed to 170 µg/L for 21 d [126]. After 3-9 d of exposure, a substantial increase in the size of lipid droplets and lipofuscin granules was observed, an indication of enhanced lipid catabolism by peroxidation. By day 21, liver lipid degeneration was observable, indicating that the detoxification system of fish may have reached exhaustion. This was supported by increased mortality rates. Fish that were exposed for only 11 d showed hepatic recovery after 18 d in clean water.

Cytopathological alterations in kidney tubules, including proliferation of smooth endoplasmic reticulum (SER), atypical mitochondria and lysosomes, as well as alterations in the apical plasma lemma were observed in rainbow trout that were exposed to 10 µg/L atrazine for 4 weeks [79]. The proximal segments (PS) of the tubules were more sensitive to such damage by atrazine than distal segments (DS), which showed signs of damage only at or above 20 µg/L. In PS II cells, typical changes included peroxisome proliferation, cup shaped mitochondria as well as altered basal labyrinth. Distal segments were characterized by mitochondria with deformed cristae, and vacuolized cell base.

Other studies also revealed ultra structural damage in kidney and hepatocytes of rainbow trout at 10-40 µg/L atrazine [127,128]. Trout are more sensitive than carp or zebrafish to sublethal stress caused by atrazine. The above studies confirmed that atrazine is not primarily excreted via the gills, but is filtered and reabsorbed by the kidneys. Chronic toxicity to leopard frog tadpoles and American toads (*Bufo americanus*) as well as rainbow trout and channel catfish was estimated based on acute exposures [82]. The NOEC for both tadpoles was predicted to be around 650 µg/L atrazine, although only survival was considered in the study. Atrazine and alachlor were synergistic in a 50:50 mixture to both tadpoles and fish. The predicted NOEC for the mixture was 150 µg/L for tadpoles and 220 µg/L for trout. The LC_1, an estimate of the chronic threshold for *R. pipiens* tadpoles, was 32.6 µg/L.

In a pioneering field study, atrazine was shown to significantly reduce the reproductive success of the common frog in a pond in Britain [129] (cited by Morgan et al. [130]). In follow-up laboratory studies, atrazine was found to reduce the amount of maternal jelly coating surrounding frog eggs [130].

Britson and Threlkeld [131] treated outdoor microcosms with 96-192 µg/L atrazine and studied the effects on grey treefrog populations over a 4 month period. Half of the microcosms were re-dosed at day 62. The number of tadpoles collected decreased with increasing atrazine concentrations. A negative correlation was also found for days to metamorphosis, tadpole size, developmental stage at collection and froglet size at metamorphosis with increasing atrazine levels. Atrazine exposure also enhanced the occurrence of malformations, namely, missing eyes in tadpoles. These authors concluded that "unless experiments are conducted to examine responses across the complete anuran life cycle, i.e., egg to egg, and with multiple interacting stressors present, little insight will be gained into how individuals and populations are affected by long-term outdoor exposure" [131]. These conclusions are supported by the field work of Bishop et al. [25] who studied the effects of agricultural chemicals on anuran populations in southern Ontario.

2.6.5 Mammalian and avian toxicity

Stevens and Sumner [2] reviewed the chronic toxicity of atrazine in mammals and stated that dietary exposure in rats and mice produces weight loss, growth retardation and reduced food consumption. Such effects are seen after dietary intake of 100-500 ppm atrazine after 6 months. Single acute oral doses resulting in mortality ranged from 1400-5100 mg/kg in rats and mice, while intraperitoneal injections produced much greater toxicity i.e., LD50 of 125 mg/kg [6]. Chronic oral doses of 100 mg/kg for 21 d or 760 mg/kg for 30 d failed to induce significant effects in cattle. On the other hand, female sheep died from daily doses of 30 mg/kg administered over a period of 36-60 d [6].

Atrazine was chosen to undergo a special review by the U.S. EPA because there is evidence of oncogenicity from animal studies [2]. To determine the potential for genotoxic effects from human consumption of contaminated water in Illinois, Taets et al. [132] examined the clastogenic properties of atrazine, simazine and cyanazine and combinations of these triazines. Because atrazine concentrations exceeded 3 µg/L (the MCL) in 25% of the water supplies tested, the investigators used this concentration in the *in vitro* studies, as well as the highest concentration of each triazine detected. Atrazine caused significantly higher occurrences of chromosomal damage at both concentrations compared to control.

Similar findings were also reported by Biradar and Rayburn [133] who also found clastogenic effects at the MCL. Both whole cell and flow karyotype tests detected chromosomal aberrations. Cyanazine did not produce significant chromosomal changes at the test concentrations, whereas simazine did so in whole cell assays only. When atrazine was combined with simazine or cyanazine, clastogenic effects occurred at the same levels or less, compared to atrazine alone. Cyanazine and simazine together proved to be non-clastogenic and a ternary mixture of the three triazines was less clastogenic than atrazine alone [132]. Chromosomal aberrations have also been detected in bone marrow cells of mice exposed to a single dose of atrazine as well as in human lymphocyte cultures treated with 100 µg/L [132].

Atrazine toxicity in birds has not been as extensively studied as in aquatic organisms because most avian species, like wild mammalian species, are unlikely to be exposed to high concentrations of this herbicide in the environment. Laboratory data suggests a high level of tolerance. In a short term feeding study, [6] northern bobwhites, Japanese quail, ring-necked pheasant and mallard ducks were studied after exposure to technical grade atrazine. The LC50 for all species was >5000 mg/kg in feed. No mortality was seen in any birds below 2500 mg/kg. Chickens ingesting 100 mg/kg atrazine in their diet had no observable toxic responses or changes in egg production or weight [6].

2.7 POTENTIAL FOR TRIAZINE HERBICIDES TO ACT AS ENDOCRINE DISRUPTORS

Our working definition of an endocrine disrupting compound:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

A substantial body of published literature exists addressing the impacts of the triazines on the endocrine system, and this is reported below under three major headings: chronic feeding studies with laboratory mammals; *in vivo* and *in vitro* studies of steroid receptor interactions; and chronic effects on the endocrine system at ecologically relevant concentrations.

2.7.1 Chronic feeding studies with laboratory rats and mice

A recently published review [134] of nine separate chronic feeding studies with mice and rats concludes that, of the tested species (Sprague-Dawley (SD) rats, Fisher 344 rats and CD-1 mice) only female SD rats showed increased incidence of tumours from atrazine exposure. Female SD rats fed atrazine at levels of 400, 500 and 1000 ppm developed mammary tumours earlier than control animals and the incidence of tumours was significantly increased from feeding at levels of ≥70 ppm in one study and 400 ppm in another study. In three other studies, however, no significant differences were seen.

Because no increase in tumour incidence was observed in ovariectomized SD rats after 24 months of atrazine treatment at 400 ppm, the observed increased incidence in previous studies appeared to be due to an acceleration of the normal reproductive aging process, resulting in increased exposure to endogenous estrogen and prolactin [134]. Therefore, it was concluded that atrazine did not act as a direct estrogenic agent, but rather, promoted mammary tumour formation indirectly by causing an earlier onset of a normal, age related hormonal state, which results in prolonged and increased exposure to endogenous estrogen and prolactin. It was further stated that high doses of atrazine effectively block the estrogen-induced surges in prolactin and luteinizing hormone (LH); disrupting the estrous cycle and exacerbating the normal aging process in SD rats.

It is well established that, as female SD rats age, hypothalamic function deteriorates and limits the reproductive lifespan of the animals [134]. With increasing age, certain non-adrenergic neurons in the hypothalamus fail to stimulate an adequate release of gonadotropin-releasing hormone. These neurons appear to be unable to respond adequately to pre-ovulatory rising levels of ovarian estrogen so that the GnRH-stimulated release of LH is delayed or absent. As a result of this absent or delayed LH surge, ovulation does not occur and the ovary remains active for extended periods of time, maintaining sustained estrogen secretion, thereby enhancing mammary tumour formation. The studies reviewed by Stevens et al. [134] demonstrate that atrazine did not act as an estrogen mimic; nevertheless atrazine caused endocrine mediated toxicity.

The mechanisms behind this disruption of hormone function were not discussed in the review; however, Barton and Andersen [135] state that the ability of the female rat or mouse brain to produce a LH surge resulting in ovulation depends on its protection from the reactive catechol metabolite of estradiol during development.

Oral administration of diaminochlorotriazine (DACT), the principal animal metabolite of atrazine, to female SD rats resulted in a greater development of astrocyte inclusion granules in the hypothalamic region [136]. These changes normally occur in aging rats, and have been shown to increase markedly when estrogens are elevated. Cooper and co-workers [137] also determined that atrazine modulates the neuroendocrine system, without mimicking steroid hormone action, by disrupting hypothalamic control of pituitary-ovarian function. Cooper and Kavlock [138] argue further that not only did their work with atrazine-exposed rats [137,139] show that the CNS control of pituitary-ovarian function was disrupted, but that pseudo-pregnancy and prolonged diestrus was observed, depending upon the dose. The exact mechanism(s) by which atrazine effects the releasing hormone have still not been defined.

Cooper and Kavlock [138] contend that the hypothesis of Stevens et al. [134] that "atrazine brings on an early onset of constant estrus and an endocrine milieu that is conducive to tumour development", is not consistent with their own observations of repetitive pseudo-pregnancies and diestrus in young adult rats. Cooper et al. [140] also state that normal aging in most strains of rats involves disrupted ovarian cycling and the absence of corpora lutea in ovaries. In their studies, however, atrazine dosed rats had large corpora lutea. The suppression of pituitary hormone secretion seems to be due to an effect on the CNS not the pituitary [140].

Wetzel et al. [141] reported an increased incidence of mammary carcinomas in female SD rats following lifetime exposure to atrazine and simazine at or above the maximum tolerated dose [142]. Eldridge et al. [142] investigated whether short term exposure to these triazine herbicides in rats would alter the estrus cycle. Treatment involved daily gavage of 100 or 300 mg/kg for 14-23 d to both SD and Fisher 344 rats (<13 weeks old). Significant reduction in body weights of both SD and Fisher female rats at both dose levels were accompanied by a significant reduction in ovarian and uterine weight and a decrease in circulating estradiol levels [142]. The magnitude of these effects was less in Fisher rats, and the effects of simazine were less pronounced than those of atrazine. SD rats showed a treatment-related lengthening of the estrus cycle with a greater percentage of d spent in estrus versus diestrus compared to controls. In contrast, while dosed Fisher 344 rats also exhibited a significant trend toward cycle lengthening, they had an increased percentage of cycle days in diestrus versus estrus [142]. The increased number of days in estrus in SD rats was characterized by a prevalence of cornified and nucleated vaginal epithelial cells. The estrus cycle disruptions observed in SD and Fisher female rats would result in a longer period of exposure to circulating estrogen and progesterone, respectively. In a lifetime feeding study [143], Wetzel et al. found that atrazine had no effect on the percent of days spent in estrus in Fisher 344 rats at a concentration of up to 400 ppm. However, SD rats spent more days in estrus from 9 through 18 months of age. This effect was significant after 9 and 18 months of treatment at 400 ppm, and after 1 and 9 months at 70 ppm.

Plasma estradiol levels were significantly elevated at 3 months in SD rats fed 70 or 400 ppm atrazine but not in Fisher rats. Prolactin levels at 9 months were also elevated in SD rats and not Fisher rats, whereas progesterone levels were not affected. There was a significantly earlier onset of mammary and pituitary tumours in SD rats fed 400 ppm during the first year of treatment, although the incidence of tumours was not significantly higher than control during the two year study. The authors stated that the occurrence of tumours appeared to be related to the neurotoxic action of estrogen on the tuberinfundibular dopaminergic neurons in the basal hypothalamus, neurons that are responsible for the secretion of prolactin. Elevated prolactin levels in rats, as seen in this study, have been linked to increased development of malignant tumours [143].

2.7.2 Short-term *in vivo* and *in vitro* studies of steroid receptor interactions

In short term, 3-d exposures, atrazine, simazine and DATC proved to be "weak estrogenic antagonists" in SD rats [144]. Estrogen-stimulated responses such as progesterone receptor (PR) levels in uterus, and thymidine incorporation into DNA, were inhibited at levels of 50-300 mg/kg/d with or without estradiol injections but not at 1-10 mg/kg/d. Atrazine, simazine and their common metabolite, DATC, had similar antiestrogenic properties, and it was concluded that their combined exposure should be used for risk assessment purposes [144]. In a follow-up article [145], Tennant et al. described the binding of atrazine and simazine to the rat estrogen receptor (ER). Ovariectomized rats were fed triazines for 2 d at 300 mg/kg. Uterine ER binding capacity was reduced by approximately 30%, although none of the triazines showed an ability to compete against binding of radiolabeled estradiol. No displacement of radiolabeled ligand binding was detected at triazine concentrations of up to 10^{-3} M over an 18 h period. Therefore, the complete responses to triazines in SD rats were thought to be due to "inhibition of events other than or in addition to ER binding of estrogen" [145].

Connor et al. [146] did not find evidence of ER mediated responses *in vivo* or *in vitro* for atrazine or simazine. The herbicides were administered to 21 d old SD rats at 50-300 mg/kg/d for 3 d. No significant induction of uterine net weight, cytosolic progesterone receptor binding or peroxidase activity was observed. Animals that also received 10 µg/kg/d estradiol showed approximately 6-, 9-, and 10-fold increases in these parameters, respectively. These responses were not significantly different from treatment with estradiol alone in the case of uterine weight. Significant decreases in uterine PR binding activity and peroxidase activity were observed for all doses of atrazine and simazine [146], with or without estradiol co-treatment.

In vitro, human breast cancer cell proliferation was not significantly altered by atrazine or simazine alone at concentrations of 0.01-10 µM [146]. Estradiol on the other hand, caused cancer cell proliferation at 1 nM with or without the co-administration of either herbicide at any of these treatment levels. Also, estrogen dependent yeast cells were not capable of growth on media supplemented with these triazines in place of estradiol. Collectively, these results indicated that the reported *in vivo* endocrine modulation by triazines are not ER mediated, based on the *in vitro* systems used [146].

Atrazine disrupted normal endocrine functioning in 90-d old Fisher rats after a 7-d exposure period to 120 mg/kg/d [147]. Both males and females showed significant body weight loss; increased weight of pituitary and prostate glands were also noted. A transiently prolonged estrus cycle was noted in this strain of rats with extended vaginal diestrus and, as a result, the rate of successful mating decreased in the first week after treatment when both sexes were exposed or when only females were exposed. Although not significantly altered, the number of pups tended to decrease with treatment and the percent of male pups was lower.

Atrazine also caused similar disturbances in the estrus cycle of pigs. The relatively low concentration of 1 mg/kg/d produced altered estradiol levels and failure of expected estrus [148]. Seven-month-old pigs were fed atrazine for 19 d before the onset of expected estrus. Two days prior to expected estrus, serum estradiol levels were significantly reduced compared to controls, while on the day of expected estrus and on the subsequent two days, estradiol levels were significantly higher. Normally, estradiol levels surge just prior to estrus: the lack of such a surge was thought to have caused the failure of the onset of estrus in treated pigs. Histopathological examination indicated a phase of uterine rest or diestrus. A delayed onset of estrus was observed on day 26 of the cycle.

Tran and co-workers [149] conducted *in vitro* studies examining the ER mediated effects of triazines with yeast cells, the results of which suggest that whether or not triazines interact with the ER depends on the concentration of estradiol present. Atrazine, cyanazine, simazine and diazinon had no estrogenic activity in yeast cells expressing human ER (hER) in the presence of an optimal concentration of estradiol (20 nM). At this estradiol level, the triazines did not reduce reporter activity linked to the hER, however, at a level of 0.5 nM, estradiol did reduce reporter activity in a dose dependent manner. Atrazine was the most potent inhibitor of estrogen dependent reporter activity, reducing it to 55% of control at a concentration of 20 µM in the presence of 0.5 nM estradiol. The order of inhibitor potency was: triazine > cyanazine > simazine > diazinon.

Significant binding to the hER was observed for all of these triazines, as evidenced by displacement of tritiated estradiol from the receptor. The order of potency was: cyanazine > atrazine > diazinon > simazine [149]. This study demonstrated that the binding of the triazines to the hER was greater than that exhibited to rat ER (see [145]).

In contrast, other studies detected little or no ER binding of triazine herbicides. For example, Balagner et al. [150] used an ER-reporter gene complex to measure the binding affinity of atrazine and other triazines *in vitro*. Their work detected no significant induction of the luciferase gene at triazine concentrations of 10^{-7} to 10^{-5} M, indicating a possible lack of binding to the ER-complex, or inactivation of it. Graumann et al. [151] did not detect changes in estradiol dependent reporter gene transactivation, and found a lack of tritiated ligand displacement from the ER. Atrazine and diazinon did not activate the ER-reporter complex singly or in combination at concentrations of 10^{-9}-10^{-4} M and no synergism was observed. The authors concluded that atrazine does not bind to the ER in the yeast assay, which is supported by the results of Soto et al. [152] who found no estrogenic activity in the E-screen assay. No explanations were given as to the source of the contradictory findings.

Vonier and co-workers [153] investigated the interaction of 23 pesticides with the ER and PR in the American alligator. Protein extracts were made from the oviducts of alligators and incubated with the contaminants and radiolabeled estradiol. Both atrazine and cyanazine competed with radioligands for binding to the ER with IC_{50} values of 20.7 and 19 µM, respectively, compared to 0.0078 µM for estradiol. The most potent ER binding was found in DDT compounds: IC_{50} values were 2.2-9.1 µM. Atrazine and cyanazine interaction with alligator PR was also significant at 30 µM. These triazines reduced radioligand binding to 66% and 62% of control levels. Although triazine binding to the ER and PR was significant, receptor agonism/antagonism was not studied. Binding activity may have been underestimated due to the presence of other binding proteins in the extracts.

Crain et al. [154] also used American alligators to investigate the endocrine disruption potential of atrazine and other environmental pollutants. They designed their study to take into consideration the fact that incubation temperature of reptile eggs is a determining factor of the sex ratio of hatchlings. Alligator eggs were treated with 0.14 -14 ppm atrazine and were incubated at temperatures producing either 100% males or 100% females. The hypothesis that atrazine increases aromatase activity in juvenile alligators was tested. Aromatase is the enzyme that metabolizes testosterone into estradiol *in vivo*. As a p450 enzyme, aromatase activity may be induced by certain xenobiotics. Increased activity may increase endogenous estradiol to harmful levels. Atrazine exposure more than doubled aromatase activity in hatchling alligators incubated at the male-producing temperature, although this was not statistically significant. No such response was seen at the lower temperature. Sex reversal was not observed. Steroid hormones and incubation temperatures exhibited synergism, with estrogen exerting greater effect at intermediate temperatures which normally produce both males and females [154].

Further evidence of atrazine-steroid hormone receptor interaction and species variability of this effect is provided by the work of Danzo [155]. Atrazine did not inhibit radioligand binding to rabbit ER at 100 μM, but it did reduce 5-α-kihydrotestesterone binding to both the androgen receptor (AR) and androgen binding protein of male SD rats (60% and 55% of control, respectively). Atrazine did not bind to human sex hormone binding globulin. As a comparison, nonylphenol did not affect AR binding despite showing the highest potency in ER binding; DDT inhibited ligand binding to both AR and ER to less than 50% of control. Binding affinity of androgen binding protein for atrazine exceeded that for nonylphenol and DDT. The ubiquitous binding of several compounds to the AR suggests that environmental mixtures may be capable of disrupting androgenic pathways [155].

2.7.3 Chronic effects on the endocrine system at ecologically relevant concentrations

A number of studies have addressed the capacity of triazine herbicides to disrupt endocrine function herbicides at concentrations that commonly occur in surface waters during application periods [156-159]. Porter and co-workers [156] investigated the endocrine, immune and behavioural effects of environmentally-relevant levels of atrazine, aldicarb and nitrate fertilizer, alone or in combinations. Males of two species of mice were administered the test chemicals in drinking water over a period of 22-103 d at a concentration of 10 μg/L for the two pesticides and 28 ppm for nitrate. The endpoints evaluated included final body mass, spleen weight, free thyroxin index, aggression score and plaque forming cell counts (PFC). Significant treatment related effects were observed for: spleen weight (atrazine-nitrate), aggression score (atrazine-aldicarb, atrazine-nitrate and atrazine-aldicarb-nitrate) and plaque forming cell count: (atrazine, atrazine-aldicarb and atrazine-nitrate) [156]. The PFC, which is an indicator of immune system function, was the most commonly disrupted endpoint and the only one that atrazine significantly inhibited singly. The atrazine-nitrate interaction was the most consistently significant effect observed for PFC. A seasonal response was detected; in general, mice were most susceptible during the winter and least susceptible in the fall, indicating that natural fluctuations in endocrine status allow for windows of high susceptibility.

Significant effects occurred more often in the case of pesticide and fertilizer mixtures than from treatment with single compounds, which emphasizes the need for standardized mixture toxicity testing including common fertilizer mixtures. Neither atrazine nor nitrate caused alterations in the thyroxin index of mice when administered singly, but their mixture did affect this endpoint. Thyroid hormones are critical for fetal brain development and are in feedback loops with corticosteroid levels and could determine an individual's disposition in adulthood [156]. The authors stressed the need for improved test methods for standard screening of neuroendocrine active environmental pollutants.

Larson et al. [157] investigated hormonal and growth responses to atrazine exposure in larval tiger salamanders. Larvae were exposed to 75 or 250 μg/L for 86 d. Corticosterone levels were significantly elevated in both control and 250 μg/L exposed larvae between stages 2 and 4 during development, but not in the 75 μg/L treatment group. A significantly higher level of corticosterone was measured in the high treatment group compared to the lower one, but not when compared to controls. Metamorphic climax was altered by both treatment levels, although in different ways. At the lower level, larvae reached stage 4 later, but at a normal weight and size. By contrast, at the higher level, larvae reached stage 4 at the same time, but at a smaller size and lower weight compared to controls. Thyroxin concentrations in plasma were significantly elevated at stage 4 from both concentrations of atrazine compared to control. Both treatment levels caused larvae to grow slower than control. The ecological effects of such sublethal responses may include reduced population densities due to longer transformation periods which increase the probability of death by predation or other factors.

Aquatic toxicity studies have shown that cladoceran fecundity and survival endpoints are not affected at atrazine concentrations below 100 μg/L. Dodson et al. [158] however, revealed that chronic exposure of *Daphnia* to very low concentrations (0.5 μg/L) of atrazine induced a shift in the population sex ratio due to increased male production, indicating sex ratio is a very sensitive, ecologically-relevant endpoint. Males are produced in stress situations, in response to such environmental signals such as shortening day length, reductions in food supply and pheromones produced in crowded populations [158]. Males typically appear during the fall, and the sex ratio is below 50%. During unfavourable conditions, sexual reproduction allows for genetic recombination, whereas during good conditions, asexual reproduction maximizes population size to be able to cope with high predation. Production of males at an ecologically inappropriate time, i.e., early summer, would reduce population growth when conditions are optimal and when the population needs to grow as fast as possible. *Daphnia* life history is strongly influenced by chemical signals in water and it is possible that cladocerans exposed to chronic atrazine concentrations may express a maladapted population response by producing males at the wrong time [158].

The ability of atrazine to cause endocrine disruption at environmentally relevant concentrations is further evidenced by the ecotoxicological experiments of Moore and Waring [159]. Atrazine significantly impacted the pheromone mediated reproductive system of male Atlantic salmon parr at the extremely low concentration of 0.04 μg/L. Atrazine not only inhibited the olfactory detection of female priming pheromones by male fish (both *in vivo* and *in vitro*) but also directly inhibited the secretion of testosterone by the testes. Male salmon were exposed to atrazine for 5 d. Electro physiological responses to a priming pheromone, (prostaglandin F_2), recorded from olfactory epithelium were significantly reduced by 2-20 μg/L atrazine. Similar results were obtained for the response amplitude to serine and atrazine. Atrazine alone did not elicit an olfactory response, suggesting fish may be unable to avoid contaminated areas.

Normally, exposure of male salmon to ovulated female fish urine causes an increase in plasma 17,20β-dihydroxy-4-pregnen-3-one levels. Exposure of males to both female urine and atrazine at concentrations of 0.04 μg/L or higher inhibited this physiological response. The priming effect of female urine on plasma testosterone and 11-ketotestosterone levels was also significantly reduced at atrazine concentrations of 3.6 and 6.0 μg/L, respectively. There was a significant reduction in the release of glucoronidated testosterone from the testes at a concentration of 6 μg/L and up, indicating a direct effect of atrazine on testicular hormone release. This was evidenced by follow-up *in vitro* studies [159].

Moore and Waring found similar effects in salmon exposed to the cholinesterase-inhibiting insecticides diazinon and carbofuran [160,161]. However, in the case of atrazine, the mode of action on the reproductive system is more complex. Unlike carbofuran, atrazine influenced both steroid metabolism and accumulation in bile, as well as testicular androgen release. The mechanism by which atrazine inhibits olfaction in fish is not known, although it was speculated to be related to inhibition of acetylcholinesterase, an effect which has been observed in some fish [162]. On the other hand, peripheral sensitivity to sex pheromones in fish is controlled by endogenous androgens and thus atrazine may affect the expression of pheromone receptors of male fish, thereby inhibiting the endocrine responses to female pheromones. Atrazine may act as an AR antagonist, as *in vitro* evidence suggests (see previous section) and may subsequently disrupt olfactory function in male salmon.

Although standard toxicity testing suggests atrazine is a relatively harmless contaminant of aquatic environments, recent experimental evidence suggests that sublethal physiological alterations in fish and other aquatic wildlife, which have traditionally not been tested for or considered in risk assessments by the regulatory community, may be causing adverse effects in wild populations.

2.7.4 Conclusion

There is abundant evidence to indicate that atrazine and the triazines generally are endocrine disrupting compounds. Atrazine disrupted the neuroendocrine system in female rats, without mimicking estrogen, by affecting hypothalamic control of pituitary ovarian-function [137,143]. Triazines were shown to generally be non-estrogenic in studies with rodent and human estrogen receptor studies, and using the E-screen assay [145,146,149-152]. However, atrazine and cyanazine competed with tritiated estradiol for binding to the alligator ER and PR [153]. Atrazine has also been shown to be an anti-androgen in rats [155]. As a P450 enzyme inducer, atrazine was shown to induce the activity of aromatase in alligators, resulting in increased breakdown of testosterone to estradiol. The increased proliferation of mammary tumours in rats caused by both atrazine and simazine is related to altered hormonal activity and can hence be considered an outcome of endocrine disruption [134,141,142,163].

Atrazine affected endocrine, immunological and behavioural responses in mice exposed chronically to environmentally relevant concentrations [156]. Such effects were observed singly or in combination with nitrate fertilizer or the anticholinesterase insecticide, aldicarb. In larvae of the endemic tiger salamander, atrazine caused alterations in plasma thyroxin and corticosterone levels, and caused delayed maturation and reduced body mass at low concentrations [157]. Atrazine caused an increase in male *Daphnia* production, resulting in an altered sex ratio, at concentrations as low as 0.5 µg/L [157].

Atrazine significantly reduced the ability of male Atlantic salmon to detect female reproductive pheromones in water and blocked subsequent physiological responses *in vivo*. Testicular androgen secretion was also reduced or blocked by atrazine. These significant adverse effects were observed *in vivo* and *in vitro* at a concentration of only 0.04-6.0 µg/L [159].

Based on the current review of the literature, we conclude that atrazine can disrupt the neuroendocrine system at concentrations which occur in the environment. However, atrazine seems to be non-estrogenic in rodent and human ER studies, but does bind to alligator ER. It is antiandrogenic and possesses several poorly understood mechanisms of toxic action on the neuroendocrine system.

2.8 RISKS TO ONTARIO ENVIRONMENTS AND RECOMMENDATIONS

Atrazine is frequently detected in surface waters of southern Ontario, often exceeding the Canadian Water Quality Guideline for the protection of aquatic life of 1.8 µg/L. The guideline was originally created based on data from short-term toxicity tests and did not consider effects of endocrine disruption. It is evident however, that endocrine disruption in aquatic organisms can occur at and below this guideline concentration. Therefore we conclude that the aquatic environment within the agricultural landscape of southern Ontario could be at risk from current use practices of atrazine. The following issues should be priorities for further evaluation of the extent and degree of risk posed by the triazine herbicides:

- need for label regulations concerning use of the triazines in areas susceptible to surface runoff to reduce entry of these compounds into the aquatic environment.
- need to implement best management practices in high risk areas incorporating buffer strips to reduce inputs

into surface waters.

- at least one species of salmonid fish is susceptible to very low concentrations of atrazine: effects on other native species and population level effects should be evaluated.
- a revised Canadian Water Quality Guideline should be considered because numerous endocrine effects in aquatic organisms have been reported around the guideline concentration.
- fish, including forage fish, and anurans in high risk areas should be monitored for the occurrence of adverse physiological, behavioural and endocrinological effects that have been observed in laboratory studies.
- laboratory studies indicate synergistic effects of triazines in combinations with other agricultural pesticides and fertilizers; further lab and field studies on the effects of the triazines in mixtures with relevant compounds should be conducted.

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3.0 GLYPHOSATE HERBICIDES

3.1 DESCRIPTION AND USE

Glyphosate (N-(phosphonomethyl) glycine), is a non-selective, systemic organophosphate herbicide, registered for use on many food and non-food field crops, as well as for many non-crop applications, including uses in forestry, home and garden use, commercial and industrial use [1]. Glyphosate was introduced by Monsanto Company in 1971; it has been registered in the U.S. since 1974 and in Canada since 1976. The Monsanto patent on glyphosate expired in the early 1990s, finally allowing production by other agrochemical companies, including Cheminova Canada and Syngenta. In Ontario, the number of registered formulations of glyphosate has increased four-fold since 1990, with 17 formulations currently registered [2].

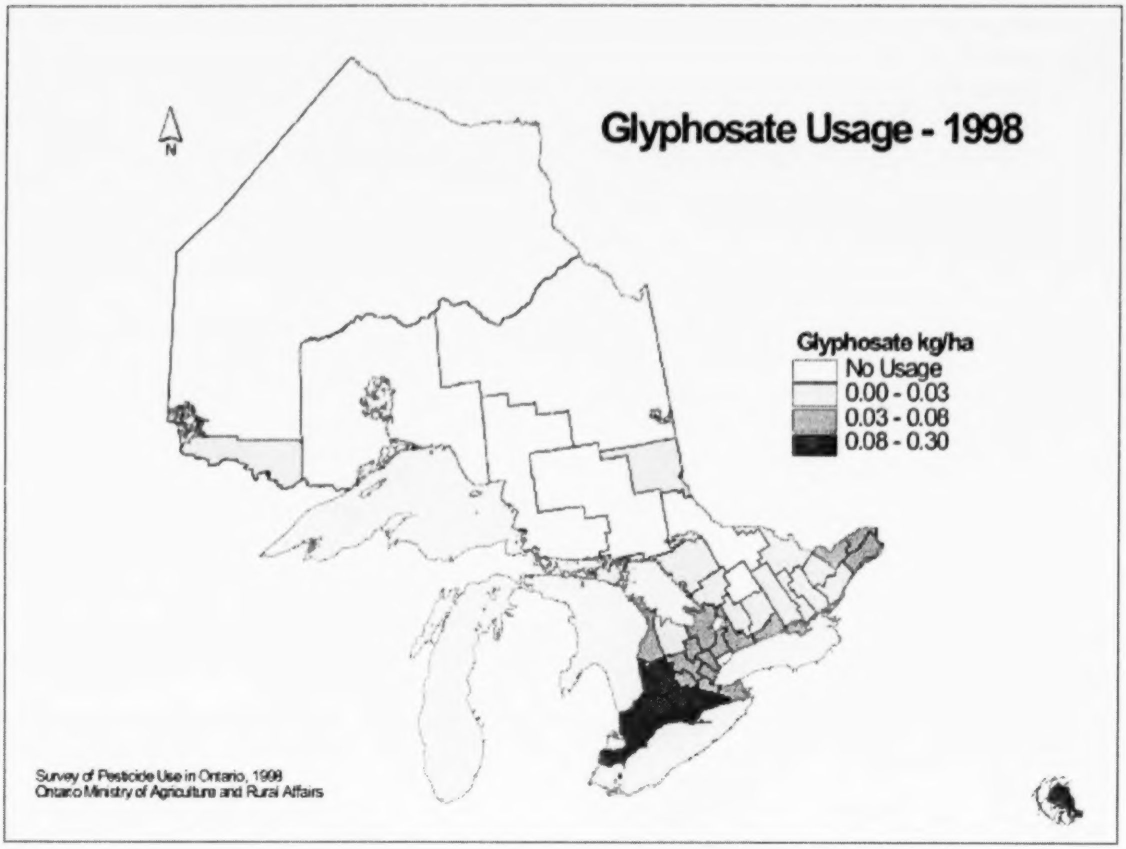
Technical glyphosate is a weak organic acid consisting of a glycine and phosphonomethyl moiety, although the active herbicidal ingredient in formulated products is one of several salts of glyphosate. These include the isopropyl amine salt, sodium salt, monoammonium salt, ethanol amine salt and sesquisodium salt. Table 3.1 lists the physicochemical properties of glyphosate. Glyphosate-trimesium is an additional active form of the herbicide, and was registered by Zeneca Agro. Technical grade glyphosate has a purity of >80%, but is generally >90%. Chemically, it is a highly polar amphoteric compound. The most heavily used formulation of glyphosate has been Roundup (the identical formulation Vision is used in Canadian forestry) containing 480 g/L of the isopropyl amine salt, which is equivalent to 356 g/L of the acid active ingredient. Many other formulations are marketed for various applications, depending on the type of crop, climate and application method. Recently, new formulations such as Roundup Biactive have been developed in order to reduce the hazards associated with surfactants traditionally used with glyphosate. Most formulations of glyphosate are solutions, although paste and soluble granules are also used. Formulations of glyphosate differ in the type and concentration of the active ingredient and the surfactants/adjuvants used. A common surfactant in the major formulation of Roundup is polyoxyethylene amine [3]. Other surfactants used in glyphosate herbicides include Ortho X-77, LI-700, R-11 and Widespread. Other additives in formulations may include sulfuric and phosphoric acid.

The volume of use of glyphosate herbicides in Ontario agriculture increased by 161% between 1988 and 1993 (158.6 to 414.8 tonnes) and by 56% between 1993 and1998 (414.8 to 647.5 tonnes), based on estimates by Hunter and McGee [4: see Figure 3.1]. The total provincial use of glyphosate is considerably higher, since the survey did not include non-agricultural usage. These use patterns combined may account for as much as 50% of the total amount used. In the U.S., during the early 1990s, home and garden use accounted for about 14% of total sales, industrial, commercial and government use was about 25% of the total and agricultural use was 61%. In the U.S. home and garden herbicide market, glyphosate is the second most widely used herbicide (3-5 million kg per year) after 2,4-D [1].

Currently, the use of glyphosate is growing at an annual rate of 20%, primarily because of the recent introduction of genetically modified crops [5]. Worldwide, 112,000 tonnes of glyphosate were used in 1998, with total sales of 1.2 billion dollars. Genetically modified crops have been grown in the U.S. and Canada since the mid-1990s. In 1995, the Canadian Food Inspection Agency approved the use of glyphosate-tolerant (Roundup Ready) soybean and canola cultivars. Monsanto Company has pioneered the biotechnology of genetically modified crops, concentrating on the introduction of a gene which causes the overproduction of insensitive ESPS synthase, the enzyme that is the primary target site of glyphosate in plants and microorganisms [6]. As a broad-spectrum contact herbicide, glyphosate could previously only be applied pre-plant to fields, to avoid any contact with the crop; however the development of glyphosate tolerance in crops allows a much greater range of uses during the growing season. In 1997, glyphosate-tolerant corn, which is also resistant to the European corn borer, and glyphosate-tolerant potatoes, which are resistant to the Colorado potato beetle and potato leaf roller virus, were registered in Canada. In 1998, there were 4.3 million ha of land planted with genetically modified crops in Canada, a threefold increase from 1997.

In Ontario, Roundup is registered for use in corn, soybean, barley, oats, wheat, potatoes and sugar beets, as well as industrial and non-agricultural areas including roadsides, pastures and recreational land [7]. Recommended application rates are 1.08-1.68 kg ai/ha for annual weeds, 1.2-5.76 kg ai/ha for perennial weeds, and 1.4-2.88 kg ai/ha for woody brush and trees. Weeds that are effectively controlled by glyphosate include annual grasses, perennial weeds (quack grass, Canada thistle, sow thistle, field bind weed, milk weed, cattails, nutsedge, poison ivy, etc.) and woody brush species.

Figure 3.1 Agricultural use of glyphosate in the Great Lakes Basin.


N
Glyphosate Usage - 1998
Glyphosate kg/ha
No Usage
0.00 - 0.03
0.03 - 0.08
0.08 - 0.30

Survey of Pesticide Use in Ontario, 1998
Ontario Ministry of Agriculture and Rural Affairs

Glyphosate has recently been registered in Canada for preharvest desiccation of crops such as wheat, barley, and rye as well as in soybeans, canola, lentils, peas, flax and forages. This type of application of glyphosate hastens the drying of crops and reduces the time between crop maturity and harvesting. Although the economic benefits of such applications are perceived to be maximized by aerial applications, only ground application is permitted to reduce off target drift and adverse effects on the environment [8]. Direct applications are used in ginseng, cherries, grapes, apples, pears, plums, peaches, and strawberries as spot treatments. The sodium salt and monoammonium salt of glyphosate are also used as plant growth regulators in some crops and in hastening the ripening of fruit [1].

Non-crop registrations include: Expedite Grass and Weed and Roundup for brush control, chemical mowing, nursery stock and roadsides. Ezject is used for tree injections in selective woody brush and tree control. Glyphosate is applied postemergence with ground equipment including ropewick and roller applications, boom sprayers, backpack sprayers and high volume equipment. The Vision formulation (360 g/L) is used exclusively in Canadian forestry operations such as site preparation and conifer release, and is the only product permitted to be applied aerially.

Glyphosate can be tank mixed by the applicator with several surfactants and other herbicides. Surfactants registered for tank mixing are mostly non-ionic compounds composed of nonyl/octyl phenoxy polyethoxylates and alkyl polyoxyethylene derivatives [2] and include Agral-90, Agsurf, Companion, Enhance, LI-700 and Sylgard 309 (Table 3.2). Herbicides that are commonly tank mixed with glyphosate include dicamba, dimethenamid, atrazine, simazine, metolachlor, flumetsulam, imazethapyr and metribuzin. Two to four of these herbicides may be co-applied depending on the weed species and crops treated.

The EPA released a Reregistration Eligibility Decision (RED) document for glyphosate in 1993, which required that additional data be supplied by the manufacturers on phytotoxicity, environmental fate, and chemistry. Only the isopropyl amine salt and sodium salt of glyphosate needed reregistration as these active ingredients were registered before 1984.

Table 3.1 Physical and chemical properties of glyphosate.

CAS #	
Technical acid	1071-83-6
Isopropyl amine salt	38641-94-0
Chemical formula	$C_3H_8NO_5P$
Molecular mass	169.1
Melting point	200 °C
Vapour pressure	1.94×10^{-7} mmHg @45 °C $<10^{-8}$ mmHg @ 25 °C
Solubility (isopropyl amine salt)	1.57% (15.7g/L)
Water organic solvents	insoluble
Log K_{ow}	-2.77 @ 20 mg/L -3.22 @ 100mg/L

Table 3.2 Formulations of glyphosate registered for use in Ontario and surfactants used in tank mixing of these herbicides [2,3].

Glyphosate formulation	Concentration (g ai/L)	Glyphosate formulation	Concentration (g ai/L)
1. Clear It 1	7	10. Roundup Dry	64% by weight
2. Clear It 2	38	11. Roundup L&G	assorted
3. Clear It 3	143	12. Roundup Transorb	360
4. Expedite Grass And Weed	147	13. Vision	356
5. Ezject	0.15 g/capsule	14. Wrangler	356
6. Glyfos	360	15. Touchdown 480	330
7. Laredo	356	16. Roundup Fast Forward Preharvest	glyphosate: glufosinate ammonium 18.7:1 volume
8. Renegade	356	17. Roundup Fast Forward Preseed	glyphosate/glufosinate ammonium 30:1 volume
9. Roundup	356		

Surfactant products commonly tank mixed with glyphosate herbicides.

Name	Type	Composition	Active (%)
1. Agral-90	Non-ionic	Nonylphenoxy polyethyoxyethanol	90
2. Agsurf	Non-ionic	Nonylphenoxy polyethyoxyethanol	92
3. Companion	Non-ionic	Octylphenoxy-polyethoxy-(9)ethanol	70
4. Enhance	Cationic with non-ionic component	Tallow fatty acid amine ethoxylate and nonylphenoxy-polyethoxy ethanol	78
5. Li-700	Non-ionic + pH adjuster acidifier	Phosphatidylcholine, methylacetic acid, alky	80
6. Sylgard 309	organosilicone non-ionic	Silicone polyether	76

Table 3.3 Selected recommended applications of glyphosate and tank mixtures with other herbicides in Ontario agriculture, 2000 [2].

Crop/use	Formulation	Application rate (formulation)
Preplant any crop Site preparation	-Roundup Transorb,	2.5-7 L/ha
	-Glyfos,	0.9-2.5 kg/ha
	-Touchdown 480,	7-12 L/ha
	-Roundup Fast Forward Preseed	2.3-4.3 kg/ha
Seed bed and inter-row weeding	-Roundup Transorb + Agral 90 or Agsurf or Enhance,	0.7-3.5 L/ha
	-Roundup Fast Forward Preseed	3-8.4 L/ha
Post harvest use	-Roundup +	1.7 L/ha
	Dicamba,	0.6 kgi/ha
	-Roundup Transorb	0.9-2.5 kg/ha
	-Glyfos,	2.5-4.3 kg/ha
	-Touchdown 480,	2.5-4.3 kg/ha
Spot treatment	-Expedite Grass And Weed	1.5-2.5 kg/ha
	-Glyfos,	2.5-4.2 kg/ha
Turf grass	-Roundup	4.7-7 L/ha (1.7-2.5 kg)
	-Glyfos	4.7-7 L/ha
	-Expedite Grass And Weed	11-17 L/ha
Weed control in ditches	-Roundup Transorb	7-12 L/ha
Field and sweet corn	-Roundup +	0.89 kg/ha
	Dimethenamid +	1-1.25 kg/ha
	Atrazine or	1.1-1.7 kg/ha
	Dicamba or	1.25 L/ha
	Dicamba + Atrazine	4.5 L /ha
	-Roundup +	2.3-2.5 L/ha
	Metolachlor + Benoxacor or	1.2-1.75 L/ha
	Atrazine	1.1-1.7 kg/ha
	-Touchdown 480 +	2.3-2.5 L/ha
	Metolachlor	1.9-2.6 kg/ha
	-Roundup Transorb	2.5 L/ha
Soybeans	-Roundup +	0.89 kg/ha
	Dimethenamid	1-1.25 kg/ha
	-Roundup +	2.5 L/ha
	Flumetsulam + Metolachlor	2.4 L/ha

	-Touchdown 480 + Imazethapyr	2.3-2.5 L/ha 0.4 L/ha
	-Touchdown 480 + Metolachlor	0.7-0.8 kg/ha 1.6-2.6 kg/ha
	-Roundup + Metolachlor + Metribuzin	2.3-2.5 L/ha 1.1-1.75 L/ha 0.6-1.45 L/ha
Apples, peaches, pears, cherries and plums	-Roundup Transorb -Touchdown 480 -Glyfos	2.25-12 L/ha 2.25-12 L/ha 2.25-12 L/ha
Grapes	-Roundup Transorb + Simazine	2.2-12 L/ha 2.5-5 L/ha
Preharvest desiccant use	-Roundup Transorb -Touchdown 480 -Glyfos	2.5-5 L/ha 2.5-5 L/ha 2.5-5 L/ha

3.2 ENVIRONMENTAL FATE AND CONCENTRATIONS

3.2.1 Soil

Glyphosate readily binds to soil, suspended organic matter and sediment particles. Laboratory studies demonstrated strong sorption in many soil types ranging from sandy loam to peat [9] (K_s = 18-377 dm^3/kg) and sandy loam to clay loam [10] (K_s = 8-138 dm^3/kg). Sorption in nine soil types was positively correlated with the unoccupied phosphate sorption capacity and not correlated with the total phosphate sorption capacity, organic matter, clay, iron or aluminum content [9]. Miles and Moye [11] suggested that the main mechanism of glyphosate sorption was by H-bonding and ion-exchange and Glass [10] correlated sorption to cation exchange capacity and clay content of soils. Sorption of glyphosate to fulvic and humic acids has been reported by several investigators [12,13]. Most of the soil bound residues of glyphosate were recovered in the fulvic acid fraction (21-33%). Humic acid complexes with polyvalent cations represent a main binding substrate for glyphosate in soils. The high affinity of glyphosate for soil particles is indicated by the lack of its desorption after 48 h of shaking with $CaCl_2$.

Field and laboratory experiments suggest that glyphosate is slightly mobile to immobile in many soils [14,15]. Leaching occurred to the extent of 0.1-6.6% of the applied activity in 30 cm columns under high water flux over 48 h. More than 90% of the activity was recovered from the upper 14 cm of eight soil types. Piccolo and Celano [16] determined that glyphosate acid forms intermolecular H-bonds between $P\text{-}O^-$ and P=O proton acceptor groups and protonated groups such as COOH, P-OH and $^+NH_2$, thereby binding to humic acids (HA) in soil and water. The water soluble HA-glyphosate complex may be translocated through soil columns depending on pH and moisture content.

In Ontario boreal forest sand soils, glyphosate demonstrated very little leaching and time until 50% disappearance (DT50) of 24 d [17]. Residue values decreased by 90% 78 d after treatment with 1.79 kg ai/ha Roundup. Residues were not detected in runoff during rainfall events. The residence time of glyphosate in soil varies with edaphic and climatic conditions; times until 50% disappearance vary from 3-174 d [3]. Microbial degradation seems to be the main environmental degradation pathway of glyphosate in both soil and water. For example, in Swedish forest soils sprayed with Roundup, the DT50 was <50 d, and was dependent on the soil respiration rate. Dissipation consisted of a fast initial phase and a much slower second phase [18]. In northern sites, 1-2% of the applied amount was recovered from soil 3 years after application.

Similarly, experiments in Finland showed that 25% of the applied amount (1.4 kg ai/ha) was recovered from sandy loam 1 year post treatment [19]. In British Columbia temperate coastal rainforest soils (alluvial sandy loam or sandy clay loam with high organic matter content), Feng et al. [20] detected 6-18% of the applied amount of glyphosate 360 d after spraying at 2 kg ai/ha. At all sampling times, over 90% of the recovered residue was in the upper 15 cm soil layer. The major metabolite of glyphosate in soil and water is aminomethylphosphonic acid (AMPA), the concentration of which increased transiently while glyphosate concentrations decreased over time. The time required for dissipation of 50% of glyphosate residues in rainforest soil was 40-60 d.

The 90% soil disappearance times of glyphosate were 30-720 d [21]; Roy et al. [22] determined a DT50 of 20 d in sandy soil planted with jack pines. Glyphosate was detectable for 35 d post-application, with most residues found in the organic top layer. Field experiments in forest soils showed that glyphosate residue disappearance was irregular during the first 4 months although 50% loss had occurred within 120 d [23]. At an Oregon site, glyphosate levels increased up to 0.15 mg/kg dry weight 180 d after application. At the same site, AMPA increased to 0.32 mg/kg within 346 d. The application rate was 4.2 kg ai/ha Accord. On a clear-cut boreal forest clay soil area with an 8° slope, glyphosate did not show loss due to runoff [22], while runoff on 6-16° slopes accounted for <1% of applied glyphosate at a rate of 1.1-3.4 kg ai/ha [24].

Newton et al. [25] conducted field studies on the dissipation of glyphosate in forest ecosystems in three states: Oregon, Michigan and Georgia. Test plots were treated with 4.12 kg ai/ha glyphosate (42%) without surfactants. This rate of application is the maximum recommended rate, about three times the normal application rates in agriculture. Glyphosate residues were highest in exposed soils and, with the exception of one higher level, concentrations peaked at or below the amount expected, and were confined to the top 15 cm layer. At the Oregon site, dense canopy reduced soil concentrations by 90% compared to exposed sites. Glyphosate was detected at a high concentration of 4.67 mg/kg in exposed soil in Michigan 14 d post-application, and declined to 0.5 mg/kg by day 330. At the Oregon and Georgia sites, glyphosate levels reached 0.15 and 1.87 mg/kg, respectively, in exposed soils. Concentrations of the metabolite AMPA peaked after 30 d or more at all sites and reached high levels of 0.32, 0.51 and 0.15 mg/kg at the Oregon, Michigan and Georgia sites, respectively. Only one soil sample showed the presence of residues (0.11 mg/kg) in the 15-30 cm layer.

Carlisle and Trevors [26] investigated the effects of glyphosate on the microbial activity of soils. The effects of glyphosate and Roundup on soil nitrogen cycling appear to be minimal except at very high concentrations. An aerobic N_2 fixation was the most susceptible activity in soils with very high inhibition occurring at 635 mg/kg, a concentration two orders of magnitude higher than that likely to be found in directly sprayed soils. Thus, at recommended application rates, glyphosate should not affect soil nitrogen cycling activities.

Overall, glyphosate binds quite strongly to soils and is therefore not susceptible to leaching; runoff into surface water may occur when glyphosate is bound to soil particles. Glyphosate is also not highly persistent in soils and disappears from the soil typically in under a year. Breakdown appears to be mainly microbial.

3.2.2 Water and Sediments

In water, glyphosate is rapidly adsorbed to suspended particulate matter and organic compounds such as humic and fulvic acids, allowing it to settle out of the water column and into the sediment. Two major pathways have been proposed for the dissipation of glyphosate in water: 1) microbial degradation to AMPA and CO_2; and 2) adsorption to sediments with subsequent microbial breakdown under anaerobic conditions [27]. Glyphosate does not undergo hydrolysis in water; Rueppel et al. [28] reported that, while AMPA was the main metabolite, it represented less than 1% of the total ^{14}C-glyphosate. Degradation did not occur in sterilized water. In Florida pond water, glyphosate dissipation followed first-order kinetics, with a DT50 of 12 d [29]. Glyphosate did not degrade to a great extent in de-chlorinated tap water with or without aeration [30]. Over a period of 78 d, 16-20% of the original amount was degraded, presumably by microbial activity. Photolysis of glyphosate in water is a minor degradative pathway. Irradiation of a solution of 1.0 mg/L glyphosate in sterile water for 1 to 14 d resulted in 18.6% to 86.7% loss, respectively [31]. Controls kept in the dark did not show degradation. Glyphosate has a longer DT50 at higher concentrations [7]. In another laboratory study, no appreciable degradation of glyphosate was observed in distilled water, while rapid degradation took place in a river water/sediment slurry [32] suggesting degradation is mainly microbial. A 35% loss of glyphosate from solution was observed immediately in river water due to adsorption to suspended sediment. Disappearance from the water column was bi-phasic, initially rapid, followed by a slower second phase.

In three small boreal forest ponds in southern Manitoba, glyphosate dissipated rapidly from surface waters, with DT50s of 3.5-11.2 d [33]. In this two year study, two ponds had been aerially treated with 0.9 kg ai/ha Roundup during the first year, whereas during the second year, ponds were treated with 2.1 kg ai/ha. The initial mean concentration of glyphosate in pond water varied from 46-83 µg/L at 2.5-h post-treatment during the second season. Dissipation of glyphosate from pond water followed first order kinetics in one pond while in another it was bi-phasic, having half-lives of 5 d for the first phase and 14 d for the second phase. AMPA levels in water increased from 0.5 µg/L on the day of treatment to 1.8 µg/L by day 64. Following spring thaw, 265 d post-treatment, residues were <0.5 µg/L. Glyphosate residues in sediments increased in all ponds to day 36, reaching a high concentration of 940 µg/kg. The maximum AMPA concentration at this time was 270 µg/kg. There was no evidence of carryover of glyphosate or AMPA in the water column between successive years. Dissipation half-lives appeared to be correlated with pond water ion chemistry, with the longest half-lives occurring in ponds with the highest divalent cation levels.

In Canadian silviculture, buffer zones of up to 100 m are established around water bodies to prevent glyphosate spray drift from entering these environments. Nevertheless, these buffers do not necessarily prevent runoff, as glyphosate residues have been detected in streams from buffered watersheds up to 21 d post-treatment [20].

Field experiments conducted by Monsanto [23], in which 4.2 kg ai/ha Accord was applied aerially on forest areas including pond and stream waters, indicate that initial surface water concentrations, which peaked at 1700 µg/L after application, declined by 50% within 7 d. Concentration of the metabolite AMPA reached 35 µg/L. Glyphosate residues in pond sediment increased up to 28 d post-treatment to a concentration of 19 mg/kg and were still measured at 1 mg/kg 400 d after application.

Feng et al. [20] investigated the fate of glyphosate in a coastal British Columbia watershed. Roundup was aerially applied at 2 kg ai/ha on 45 ha of forest, directly overspraying two tributaries and observing buffers around other streams. Residues of glyphosate in an exposed, unvegetated stream peaked at 162 µg/L 2-h post-treatment, whereas in a vegetation covered stream, they reached only 1.5 µg/L. In both tributaries, glyphosate concentrations increased 100 fold after the first rainfall, 27 h after application. Movement of the herbicide with runoff was likely increased by steep slopes. Glyphosate dissipated to less than detection levels in streams within 4 d. Stream water residues in buffered tributaries were largely undetectable with the exception of a few samples (2.47 µg/L) by 10 h after spraying. The highest residue levels observed in the aquatic ecosystem were those associated with bottom sediments of oversprayed tributaries. Residues varied in the two oversprayed streams from 0.58 to 6.8 mg/kg dry weight. Between days 196 and 364, stream sediment residues varied from 0.14-1.92 mg/kg. Off target deposits were minimal at a distance of 23.1 m from the treated area (176 mg/ha). A 10 m wide vegetative buffer zone effectively reduced the entry of glyphosate into streams in this study.

Newton and co-workers [25] also determined the aquatic fate of glyphosate in a forest field study. Glyphosate residues in pond water peaked at 1-2 mg/L after application and declined to 10 µg/L within 2-10 d. Stream water received similar initial concentrations (1.2 mg/L) but these declined much more rapidly, reaching 48 µg/L within the first day. Sediment bound glyphosate levels reached 0.69 mg/kg in streams, and became non-detectable by 335 d post-treatment.

Smith et al. [35] examined the vertical translocation of glyphosate into groundwater in Newfoundland.

Three 1 ha forest plots were treated with 13 L/ha Roundup and two were re-treated a month later with 12 L/ha. Well water at two of the stations contained no detectable levels of glyphosate throughout a 37 week monitoring period. At the third station, glyphosate was detected within two weeks of the first spraying at 25 μg/L. One week after the second spraying concentrations increased to 45 μg/L and subsequently declined to 15 μg/L over 37 weeks. The soil foundation at the site where residues leached into the water table was found to be a well drained moderately coarse textured glacial till, on a limestone bed. The other two sites had soil constricting layers, a loamy subsurface horizon with a high bulk density, which would have greatly reduced herbicide migration into groundwater.

Payne et al. [36] studied the amount of off-target deposition from aerial applications of glyphosate. Three different types of applicator equipment were compared. Glyphosate was applied as Vision, 2.1 kg ai/ha with Microfoil, ThruValve boom sprayers and D8-46 type applicators over forest plots. Down wind deposition rates at 50 m were 380, 1960 and 13400 μg/m^2 respectively, whereas at 100 m down wind they were 103, 195, 536 μg/m^2, respectively. A buffer width of 25 m around waterbodies was deemed reasonable for applications using the Microfoil and ThruValve boom sprayers, whereas 30 m was calculated as necessary for use with D8-46 equipment. In a similar experiment, Marrs et al. [37] evaluated the drift of glyphosate and the width of buffer zones needed to protect non-target plant seedlings. Glyphosate was applied at 2.2 kg ai/ha using ground equipment. Survival of potted seedling indicated that at least a 20 m wide buffer zone was necessary.

Immediately after aerial spraying of a lake with 0.75 kg ai/ha glyphosate, water residues were less than 700 μg/L and were not detected 1 hour post-treatment [38]. First flow-through water from irrigation canals treated 158-172 d earlier with 5.6 kg ai/ha glyphosate, did not contain measurable levels of the parent compound or its metabolite AMPA. However, soil samples collected prior to filling of canals contained 350 μg/kg glyphosate and 780 μg/kg AMPA [39]. A maximum glyphosate concentration of 5153 μg/L was detected in runoff from a watershed treated with 8.96 kg ai/ha herbicide the previous day [24]. This concentration decreased to < 2 μg/L within two months of application.

Glyphosate has not been routinely analysed in pesticide monitoring programs of agricultural and urban landscapes in North America, as it requires different analytical techniques from the pesticide scans used in most monitoring. Therefore, there is limited information on its occurrence/persistence in surface or groundwater, except as measured in specific studies on its fate, as described above. In 2000 however, Struger et al. [40] measured concentrations of in-use pesticides in urban and agricultural streams on the Canadian portion of Lake Ontario, including glyphosate in their analysis for the first time. In 74 samples taken, neither glyphosate nor its metabolite, AMPA, were detected, with detection limits of 50 μg/L. Thus, no samples exceeded the Canadian Water Quality Guideline for the protection of aquatic life of 65 μg/L [41]. Nevertheless, given the propensity of glyphosate to partition into the sediment, this may be the more useful and revealing sample substrate, rather than surface water itself.

3.3 BIOCONCENTRATION AND METABOLISM

3.3.1 Plants

Treated leaves of potatoes absorbed glyphosate and translocated it to the apical meristem and the roots. About 45% of the absorbed activity was recovered from the leaves and 5% from the tuber. Younger plants translocated glyphosate to a greater extent than older ones [42].

Uptake in raspberries was 9% of the amount deposited on the leaves after treatment with 2 kg ai/ha [43]. Uptake by blueberries was 14%, while most of the applied dose was recovered in the washings. Absorbed and washable amounts together were reduced by 50% within 13 d in raspberries and 20 d in blueberries. Metabolism of glyphosate in plants occurred only to a minor extent as AMPA concentrations were less than 1.5% of the parent compound.

In the British Columbia field trials of Feng et al. [20], glyphosate dissipated relatively rapidly from foliage treated with 2 kg ai/ha Roundup. Immediately after treatment, concentrations in leaf tissue were 261 µg/g in red alder (*Alnus rubra*) and 448 µg/g in salmonberry (*Rubus spectabilis*). Initial leaf litter residues were 12.5-19.5 µg/g 15 d after application about 10 fold lower than foliage residues. The 50% disappearance time was estimated to be 8-9 d in leaf litter.

In a Finnish field study, Roundup applied at 0.25-2.2 kg ai/ha, was undetectable in wild berries (*Vaccinium myrtillus*) within 1 year [44]. In contrast, glyphosate persisted in reindeer lichen (*Cladonia rangiferina*): by 270 d post-application of 0.8 kg ai/ha herbicide, glyphosate and AMPA concentrations were 45 and 2.1 mg/kg and at 390 d the corresponding values were 6.4 and 0.3 mg/kg, respectively.

Radiolabeled glyphosate disappeared from water within 3 d in outdoor experiments, while residues in water hyacinth (*Eichhornia crassipes*) were constant for the 14 d of the study [45].

3.3.2 Animals

Glyphosate would not be expected to bioaccumulate due to its high polarity (log $K_{ow} = -2.77$) and anionic character [3,7]. A maximum bioconcentration factor (BCF) of 1.6 was reported for bluegill sunfish exposed to 0.6 mg/L for 28 d [29]. Channel catfish (for Latin names of vertebrate species see Appendix 3), largemouth bass and rainbow trout exposed to 10 mg/L glyphosate for 14 d had BCFs of 0.18, 0.04, and 0.03, respectively.

After a 12-h exposure to 0.02-2 mg/L of the isopropyl ammonium salt of glyphosate, no residues were detected in rainbow trout tissue or eggs. However, upon exposure to 2 mg/L Roundup, tissue and eggs contained 60 and 80 µg/kg of glyphosate, respectively [46], indicating a BCF of 0.04.

Caged coho salmon fingerling did not contain detectable residues of glyphosate or AMPA 2 h post treatment of a forest stream at 3.3 kg ai/ha. Peak concentrations in water reached 270 µg/L [47]. The highest concentration of glyphosate in small mammals (deer mice) was 5.1 mg/kg.

In a static test, channel catfish were exposed to 0.94-0.99 mg/L glyphosate for 10 d [48]. Of the absorbed amount, 76% was recovered in the viscera. More than 90% of these extractable residues was identified as glyphosate and less than 2% as AMPA. After a 10-d period in clean water, 80% of the absorbed activity was eliminated from fish. The BCF was estimated to be 0.27.

In contrast, a far higher BCF was measured in carp using radiolabeled glyphosate, comparing radioactivity in water relative to that in the fish; Wang et al. [45] determined a BCF of up to 42.3 after 7 d exposure to 0.05 ppm glyphosate.

The metabolic fate of ^{14}C-labeled glyphosate was investigated in Sprague-Dawley rats [49]. Rats were orally administered 10 mg/kg body weight glyphosate, a concentration which had no observable toxic effects. Of the administered dose, 35-40% was absorbed from the gastrointestinal tract. Urine and feces were equally important routes of elimination. Within 7 d, the total body burden was 1% of the administered dose and was associated with the bone. Nearly 100% of the body burden of radioactivity was present as unmetabolized parent compound. Seven h after ingestion, 40% of the dose had been eliminated in the urine.

3.4 TOXIC MECHANISM OF ACTION

Glyphosate inhibits the activity of 5-enolpyruvyl shikimic acid-3-phosphate (EPSP) synthase, an enzyme of the shikimic acid pathway found in plants, fungi and prokaryotes [50]. The inhibition of this enzyme results in the cessation of synthesis of aromatic amino acid, hydroxy phenolic compounds and chlorophyll, followed by reduced protein synthesis, growth and premature cell death. EPSP takes part in the biosynthetic pathway of tryptophan, phenylalanine, and tyrosine via the conversion of shikimate to chorismate and its conversion to anthralinate [51]. These amino acids are needed for the synthesis of alkaloids, the phytohormone auxin (indole-3-acetic acid) and lignin among others [52]. At high concentrations (>55 mg/L) Roundup inhibited photosystem I and II in isolated chloroplasts, suggesting electron transport inhibition although this effect may have been due to the surfactant polyoxyethylene amine [53]. Duke and Hoagland [54] suggested that the chelation of the cations Ca^{2+} & Mg^{2+} by glyphosate may be the actual mechanism of the inhibition of ESPS.

3.5 ACUTE TOXICITY

3.5.1 Phytoplankton

Technical grade glyphosate inhibited the conversion of shikimate to chorismate in *Aerobacter aerogenes* at concentrations of >0.2 mg ai/L. Synthesis of chlorophyll-a and b and carotenoids was significantly inhibited in the green alga *Chlorella pyrenoidosa* by 55 mg/L Roundup during a 2-d exposure [53]. Periphytic algae collected from forest ponds had 4-h EC50 values of 243-479 mg/L Roundup for carbon fixation [55].

Saenz et al. [56] compared the acute effects of technical glyphosate to that of the formulated product Rondo in two species of algae. *Scenedesmus acutus* and *S. quadricauda* were exposed to 2.5-40 mg ai/L each of glyphosate and Rondo for 96 h. The formulated product caused significant inhibition of growth in both species at test concentrations of >10 mg/L. For the formulated product, the EC50s were essentially identical, 9.08 and 9.09 mg/L, in the two algae, whereas the LOECs (4.08 and 2.5 mg/L) and NOECs (3.2 and 1.25 mg/L) differed between species. Technical glyphosate had a greater impact on *S. acutus*, inhibiting growth by 77% versus 60% for *S. quadricauda* at a concentration of 12 mg/L. However, the EC50 values would suggest that *S. quadricauda* is more sensitive (EC50 = 7.2 mg/L) versus *S. acutus* (EC50 = 10.2 mg/L). The NOEC values for *S. acutus* and *S. quadricauda* were 2 and 0.77 mg/L, respectively. The formulated product was, in this case, slightly less toxic than the technical material to *S. quadricauda* (LOECs: 1.5 versus 2.5 mg/L). Significant reductions in chlorophyll-a were seen in *S. quadricauda* exposed to 50 mg/L glyphosate.

Peterson et al. [57] calculated a worst case scenario EEC of 2.848 mg ai/L for glyphosate in shallow water directly oversprayed with 4.2 kg ai/ha. This concentration exceeds the LOEC for *S. quadricauda* and allows for a safety margin of less than one order of magnitude for acute toxicity. Several species of algae, cyanobacteria and duckweed (*Lemna*) were exposed to this EEC using formulated glyphosate (Roundup). The algae *Cyclotella meneghiana* and *Nitzschia* were adversely affected by Roundup at this concentration, causing a 73% and 77% reduction in ^{14}C uptake. *Scenedesmus* and *Selenastrum* were not significantly affected. The cyanobacterium *Aphanizomenon flos-aqua* also showed a 74% reduction in ^{14}C uptake, while *Lemna minor* (a macrophyte) showed no effect for this endpoint after a 7-d exposure period. It was noted by Peterson et al. [57] that although Roundup had no effect on ^{14}C uptake in *Lemna* in their laboratory exposures, where glyphosate was dissolved in water (4.2 kg ai/ha), in a previous study, [58] glyphosate caused a complete kill of *Lemna* when oversprayed on the water surface at only 0.8 kg ai/ha. Cooley and Foy [59] noted a 37% reduction in growth as measured by dry weight in *Lemna* exposed to 8.5 mg/L glyphosate dissolved for 7 d.

Carbon fixation in periphytic algal communities collected from forest ponds was unaffected at concentrations of <0.89 mg/L Roundup in a 4-h exposure [55]. At 8.9 mg/L, carbon fixation was 82% of control level, while at 89 mg/L it was 20% of control. EC50s for three different ponds were estimated to be 35.4-69.7 mg/L.

Gardner et al. [60] reported a 96-h EC50 value of 74 mg ai/L (Rodeo) for green algae. It was noted that the pH of the test media decreased from 7 to 4.5 during the exposure. *Ankistrodesmus* sp. cell density significantly decreased at exposures of >80 mg/L, but recovered by d 10 at 80 mg/L, although not at higher concentrations. When the experiment was repeated at neutral pH, the 96-h EC50 was found to be 412 mg/L, indicating that the Rodeo formulation caused high acidity in the test solution and may have influenced the effects on the algae. In field applications, *in situ* pH changes in water are not expected.

The growth of the green alga *Chlorella sarokiniana* was inhibited by 3 mg/L glyphosate, while growth of blue green algae was 50% reduced at 2 mg/L [7]. Roundup reduced oxygen evolution by 50% in *Selenastrum capricornotum* at a concentration of only 10 µg/L in a 24-h assay [61]. A 50% reduction in algal biomass was observed at 3.83 µg/L in a 2-3 week EPA bottle test, however, when Roundup was mixed with natural stream water, concentrations ranging from 0.036-36 µg/L failed to elicit a 50% inhibitory response in either test system [61].

3.5.2 Aquatic macrophytes

Christopher and Bird [62] exposed water milfoil (*Myriophyllum spicatum*) to the Roundup formulation in a 5-d bioassay. The number of branches produced was inhibited at >1 mg/L glyphosate and the number of leaves was significantly lower at exposure concentrations >2.5 mg/L. On the other hand, 2.5 mg/L caused the formation of more buds than control. Some cultures showed browning of older leaves, yellowing and swollen nodes at 10 mg/L. Abnormal new leaves and branches were found at levels of >2.5 mg/L, however, there was no significant difference in production of roots up to 100 mg/L. These investigators compared the effects of four herbicides and noted that glyphosate was found to affect leaves and branches at lower concentrations than atrazine (>2.5 versus >20 mg/L, respectively).

Lemna gibba showed chlorotic areas in newly developing fronds within 48 h of exposure to 169 mg/L glyphosate, which inhibited growth by 30% [63]. A concentration of 16.9 mg/l was also found to cause similar damage. Electron microscopic examination revealed damage to chloroplast, mitochondria and cell walls.

Glyphosate is one of several herbicides proposed in the U.S. for control of purple loosestrife in wetlands. Accordingly, Gardner and Grue, [64] investigated the ecological impact of this herbicide in wetlands. The floating macrophyte, *Lemna gibba,* did not show significant differences in number of fronds until 48 h post-treatment with 1L/ha Rodeo + the non-ionic surfactant LI-700. This treatment resulted in mean water concentrations of 0.02 mg/L in one wetland plot and 0.06 mg/L in another, with maximum concentrations of 0.1 mg/L glyphosate. The fact that the treatment caused significant toxicity in *Lemna* may well be due to the presence of the surfactant and the fact that glyphosate came into contact with the plant leaves directly as opposed to dissolved in water as is usually done in laboratory tests.

3.5.3 Invertebrates

Technical glyphosate is generally slightly toxic to aquatic invertebrates, with LC50 values of >50 mg/L. Formulations of glyphosate are moderately to slightly toxic with 2-d EC50 values of 5.2-5600 mg/L and 21-d MATCs of 1.4-4.9 mg/L (IPCS, 1994). The higher toxicity of Roundup is due mainly to the presence of surfactants [65,66]. The 48-h LC50 of Roundup to water fleas (*Daphnia magna*) was 24 mg/L product with aeration and 37 mg/L without aeration [67].

The addition of 50 mg/L of bentonite clay to a test solution decreased the 48-h EC50 value for *Daphnia pulex* from 16 to 7 mg Roundup/L [68]. Ingestion of particle bound Roundup may have caused the higher toxicity. Immature water fleas were more sensitive to Roundup than adults.

The Rodeo formulation of glyphosate is registered for aquatic plant control, and is used by waterfowl managers to create open water habitats in wetlands. Henry et al. [69] exposed five species of invertebrates in the laboratory to determine the effects of Rodeo, the surfactant X-77 Spreader and the drift control agent ChemTrol. The chemicals were studied individually as well as in mixtures, since they are commonly tank mixed by applicators to control cattail (*Typha*) in wetlands. X-77 Spreader was the most toxic compound (up to 136 times more toxic than Rodeo) and ChemTrol was the least toxic (24 times less toxic than Rodeo). Of the species tested - *Daphnia magna*, *Hyallella azteca* (amphipod), *Chironomus riparius* (midge) and *Nephelopsis obscura* (leech) - daphnids were significantly more sensitive to Rodeo and X-77 Spreader. The other species were equally sensitive to Rodeo, but amphipods were more sensitive than midges and leeches to X-77 Spreader. The LC50 values for Rodeo ranged from 218 mg/L (equivalent to 117 mg glyphosate/L) for *D. magna* to 1261 mg/L for chironomids (see Table 3.4). The corresponding values for X-77 Spreader are 2 and 10 mg/L, and the LC50 of ChemTrol was >28,000 mg/L for all species.

Studies on the mixture toxicity of the three chemicals indicated that daphnids were most sensitive, and amphipods were intermediate in sensitivity. The joint toxic action of the mixture was additive for amphipods and midges and greater than additive for leeches, whereas in daphnids it was less than additive. The results indicated that Rodeo and X-77 spreader contributed to the acute toxicity of the mixture in proportion to their individual toxicities. Generally, the toxicity of X-77 was two orders of magnitude greater than that of Rodeo. The ternary mixture was more toxic to invertebrates than Rodeo alone, due to the higher toxicity of X-77, which contributed 75% of the toxic action. Similarly, Folmar et al. [46] found that the surfactant in the Roundup formulation was the primary toxic agent, not glyphosate. Crayfish (*Procambarus* sp.) are tolerant of very high concentrations of glyphosate. A 96-h LC50 of 64,000 mg/L Roundup was determined by Abdehgani et al. [70]. The toxicity of the anionic surfactant Syndets (80% polyethylene glycol alky ether) was much greater (LC50 of 19 mg/L).

Servizi et al. [65] compared the toxic effects of Roundup with that of the surfactant Mon 0818 which is found in the Roundup formulation at a concentration of 15%. The 96-h LC50 was 25.5 mg/L in *D. pulex* , whereas that of the surfactant was 2.0 mg/L. Technical grade glyphosate is much less toxic to daphnids (LC50 range of 700-1000 mg/L).

Drift response of stream invertebrates was not significantly affected by aerial treatment of forest plots with Roundup at 2.0 kg ai/ha. This treatment resulted in peak concentrations of 162 µg/L which was reduced to 50 µg/L within 10 hours [71]. However, it was noted that *Gammarus* sp. and *Paraleptophlebia* sp. (mayfly) showed increased drift after application. The numbers of gammarids collected in a Carnation creek (British Columbia) tributary were about twice as high on the night of the application of Roundup as the day before. The EC50 for this group was 43 mg/L Roundup [46]. The abundance of *Paraleptophlebia* sp. in drift nets increased about 10 fold after application, indicating that this mayfly may be significantly more sensitive than *Ephemerella* sp., which showed no avoidance at concentrations below 1 mg/L Roundup in laboratory studies [72].

3.5.4 Fish and Amphibians

There is a large body of evidence to indicate that the toxicity of glyphosate herbicides to vertebrates is associated with the surfactants present in the formulations [3,7,73]. Roundup is generally at least an order of magnitude more toxic than technical glyphosate to fish (Tables 3.5 and 3.6). For example, the 96-h LC50s of the surfactant MON 0818, glyphosate and Roundup in rainbow trout are 2.0 mg/L, 86 mg/L and 8.3 mg/L, respectively [74].

Other factors which have been shown to influence the toxicity of glyphosate in laboratory studies include water hardness, pH and temperature. Wan et al. [73] reported that the toxicity of technical grade glyphosate to salmonids increased as hardness and pH decreased, whereas for Roundup and Accord the opposite was true. These results are in accordance with that of Mitchell et al. [66] and Folmer et al. [46] who also showed similar trends in bluegill sunfish. Roundup became more toxic to both species as pH increased from 6.5-7.5, at this level the effect of pH stabilized.

Wan et al. [73] exposed coho, chum, chinook and pink salmon as well as rainbow trout to technical glyphosate, MON 0818 (75% tallow amine) surfactant, MON 8709 (similar to Roundup with a 10% MON 0818 content instead of 15% v/v) and Roundup. The surfactant MON 0818 was found to be the most toxic of the test chemicals, having an LC50 of 2.5 mg/L for rainbow trout. The corresponding values for Roundup and glyphosate were 15 and 22 mg/L, respectively. Glyphosate was most toxic to fish in soft water, whereas MON 0818 was most toxic in hard water (see Table 3.6). Similarly, Roundup was significantly more toxic in hard water, and about two times as lethal as MON 8709. Water hardness especially, but also pH, appear to be key factors in determining the toxicity of glyphosate and Roundup to salmonids. These findings are well corroborated by the results of Servizi et al. [65] with similar salmonid species.

Wan et al. [75] investigated the effects of a herbicide spray marker (dye) Basacid blue (NB 755) on the toxicity of Roundup in fish. The dye did not alter the acute effects produced by Roundup alone in soft or hard waters. As in the previous study by these authors [73], toxicity increased as hardness increased with or without the dye.

No significant differences in susceptibility were detected between rainbow trout, coho and chinook salmon after exposure to Roundup [66]. 96-h LC50 values were 26, 22 and 20 mg/L Roundup. The authors also listed the isopropyl amine salt equivalent values as 12, 7.4 and 11 mg/L, respectively. A mixture of Rodeo and X-77 surfactant proved to be much less toxic to fish (10-fold), having LC50s of 680, 750 and 1000 mg/L. Rodeo alone was the least toxic of the three chemicals (EC50 = 1100 mg/L).

Monsanto has recently introduced a new formulation of glyphosate, Roundup-Biactive, which has been found to be practically non-toxic to aquatic organisms due to the use of different surfactants [76]. These investigators compared the acute effects of Roundup, Touchdown, Roundup-Biactive, technical glyphosate (isopropylamine salt), and glyphosate acid to six species of Australian frogs (Table 3.4). Roundup proved to be the most toxic formulation to tadpoles of four species, with LC50 values of 8.1-32.2 mg/L. Touchdown was slightly less toxic, (LC50 = 27.3-48.7 mg/L) followed by Roundup-Biactive (LC50 = 911-1000 mg/L) and the isopropylamine salt of glyphosate, which did not cause mortality at 500-684 mg/L. The new formulation of glyphosate was 100 times less toxic to the most sensitive species than Roundup.

In southern Ontario an increased incidence of chromosomal damage was noted in tadpoles collected from agricultural areas [77]. Upon exposing bullfrog tadpoles to Roundup for 24 h, Clements et al. [78] detected a significantly higher occurrence of DNA damage at concentrations of 6.5 mg/L or higher. A low concentration of 1.6 mg/L had no significant effects on DNA structure, although at higher levels there was a strong correlation between the incidence of DNA damage and Roundup concentration. Wood frog embryos collected from forest ponds oversprayed with Vision at 1.44 kg ai/ha the previous August, had reduced hatching success and a higher rate of post-hatch deformities [79].

3.5.5 Laboratory mammals and *in vitro* systems

Glyphosate and its formulations have very low acute toxicities by oral and dermal routes, although they are more toxic intraperitoneally. The oral LD_{50} of glyphosate in rats is 2-5 g/kg bw and that of Roundup is >5 mg/kg bw. Olorunsaga [80] observed dose related reduced respiration control and increased phosphatase activity in rat mitochondria at concentrations ranging from 15-120 mg/kg bw. This effect was also observed in rat liver mitochondria *in vitro* and it was suggested that acute toxicity at lethal doses may occur due to the uncoupling of oxidative phosphorylation.

Martinez et al. [81] compared the effects on rats of glyphosate, Roundup and the surfactant Mon 0818. Both glyphosate and Mon 0818 produced pulmonary toxicity from either oral or intra tracheal administration. The toxicity of Roundup was greater than can be accounted for on the basis of the dose response data from either compound alone.

Adam et al. [82] exposed rats intratracheally to glyphosate (200 mg/kg), the surfactant POEA (100 mg/kg) or Roundup. The test animals showed immediate respiratory effects which were more severe and lasted longer in the POEA exposed groups, which showed bloody nose secretions and lung haemorrhages.

It has been stated that technical glyphosate is not mutagenic in Ames tests, and does not produce chromosomal aberrations *in-vivo*, (reviewed by IPCS [3]). However, Bolognesi et al. [83] did observe DNA damage (single strand breaks) and a significant increase in chromosomal alterations both *in vitro* and *in vivo* in CD-1 mice exposed to Roundup or glyphosate. Both chemicals produced an increase in micronucleus frequency, sister chromatid exchange in human blood cells, and DNA breaks. Roundup had a higher potency in causing such effects.

3.5.6 Birds and Mammals

Zebra finch are more susceptible to Roundup than rodents, based on the work of Evans and Batty [84]. All birds died within 3-7 d when allowed access to seed containing 5 g/kg glyphosate as Roundup, although the deaths may have been caused by starvation, since food intake was drastically reduced. Finches eating seed treated with 2.5 g/kg glyphosate survived the 5-d study period without a reduction in food intake or any overt ill effects. Previous work [85] indicates that the acute toxicity is equally low in other species of birds; for example Japanese quail, Northern bobwhite and mallards, all have LC50s of >4.6 g/kg diet.

Table 3.4 Acute toxicity of glyphosate herbicides to aquatic organisms. Vertebrate and invertebrate species indigenous to Ontario are identified by an (I) after the species name.

Species	Common name	Chemical formulation	Exposure methoda	Test time	EC50 (mg/L)	Reference
Phytoplankton						
Scenedasmus acutus	green alga	Technical	Static	96-h	10.2	[56]
Scenedasmus acutus	green alga	Roundup	Static	96-h	9	[56]
Scenedasmus quadricauda	green alga	Technical	Static	96-h	7.2	[56]
Scenedasmus quadricauda	green alga	Roundup	Static	96-h	9	[56]
Selenastrum capricornutum	green alga	Roundup	Static	96-h	7.8	[86]
Selenastrum capricornutum	green alga	Technical	Static	96-h	7.8	[87]
Selenastrum capricornutum	green alga	Sting	Static	96-h	1.0	[88]
Chlorella pyrenoidosa	green alga	Roundup	Static	2-7 d	>55	[53]
Skeletonema costatum	green alga	Technical	Static	96-h	1.2	[89]
Invertebrates						
Daphnia magna (I)	water flea	Roundup	Static	48-h	5.3	[67]
Daphnia magna	water flea	Roundup	Static	48-h	7.3	[46]
Daphnia magna (I)	water flea	Rodeo	Static	48-h	218	[69]
Daphnia magna (I)	water flea	Rodeo	Static	48-h	61.7	[90]
Daphnia pulex (I)	water flea	Roundup	Static	96-h	7.9	[68]
Daphnia pinulata	water flea	Rondo	Static	48-h	66.2	[90]
Nephelopsis obscura (I)	leech	Rodeo	Static	96-h	1177	[69]
Mysidopsis bahia	shrimp	Technical	Static	96-h	>1000	[89]
Procambarus sp.	crayfish	Roundup	Static	96-h	64000	[70]
Hyalella azteca (I)	isopod	Rodeo	Static	96-h	720	[69]
Chironomus plumosus (I)	midge	Technical	Static	48-h	55	[46]
Chironumus plumosus (I)	midge	Roundup	Static	48-h	44	[46]
Chironomus riparius (I)	midge	Rodeo	Static	48-h	1216	[69]
Fish						
Salmo trutta (I)	brown trout	Roundup	Static	96-h	4.5	[91]
Cyprinus carpio	*carp*	*Technical*	*Static*	*96-h*	*620*	*[92]*
Oncorhynchus kisutch)	*coho salmon*	*Roundup*	*Static*	*96-h*	*13*	*[73]*
Oncorhynchus kisutch	coho salmon	Roundup	Static	96-h	15	[66]
Oncorhynchus kisutch	coho salmon	Rodeo + X-77	Static	96-h	1000	[66]

Oncorhynchus kisutch	coho salmon	Roundup	Static	96-h	42	[65]
Oncorhynchus keta	chum salmon	Roundup	Static	96-h	11	[73]
Oncorhynchus tshawytsha	chinook salmon	Roundup	Static	96-h	17	[73]
Oncorhynchus tshawytsha	chinook salmon	Rodeo + X-77	Static	96 h	750	[66]
Oncorhynchus gorbuscha	pink salmon	Roundup	Static	96-h	14	[73]
Oncorhynchus mykiss	rainbow trout	Technical	Static	96-h	148-211	[73]
Oncorhynchus mykiss	rainbow trout	Roundup	Static	96-h	14	[73]
Oncorhynchus mykiss	rainbow trout	Roundup	Static	96-h	25	[65]
Oncorhynchus mykiss	rainbow trout	Roundup	Static	96-h	20-26	[66]
Oncorhynchus mykiss	rainbow trout	Rodeo	Static	96-h	1100	[66]
Oncorhynchus mykiss	rainbow trout	Rodeo + X-77	Static	96-h	680	[66]
Oncorhynchus nerka	sockeye salmon	Roundup	Static	96-h	27	[65]

Amphibians

Lymnodynastes dorsalis	obblebonk Frog tadpole	Technical	SR	48-h	>400	[76]
Litoria moorei	Western Green Tree Frog tadpole	Technical	SR	48-h	>343	[76]
Heleioporus eyrei	Moaning Frog tadpole	Technical	SR	48-h	>373	[76]
Crinia insignifera	Brown Froglet tadpole	Technical	SR	48-h	>466	[76]
Lymnodynastes dorsalis	Pobblebonk Frog tadpole	Roundup	SR	48-h	3	[76]
Litoria moorei	Western Green Tree Frog tadpole	Roundup	SR	48-h	2.9	[76]
Heleioporus eyrei	Moaning Frog tadpole	Roundup	SR	48-h	11.6	[76]
Crinia insignifera	Brown Froglet tadpole	Roundup	SR	48-h	6.3	[76]
Lymnodynastes dorsalis	Pobblebonk Frog tadpole	Roundup Biactive	SR	48-h	>400	[76]

Litoria moorei	Western Green Tree Frog tadpole	Roundup Biactive	SR	48-h	328	[76]
Heleioporus eyrei	Moaning Frog tadpole	Roundup Biactive	SR	48-h	>427	[76]
Crinia insignifera	Brown Froglet tadpole	Roundup Biactive	SR	48-h	>494	[76]
Lymnodynastes dorsalis	Pobblebonk Frog tadpole	Touchdown	SR	48-h	12	[76]

[a] SR = Static Renewal

Table 3.5 Comparison of the acute toxicity of glyphosate, Roundup formulation and the surfactant used in Roundup (from [7]).

Organism	Chemical	Exposure time	LC50 (mg/L)
Rainbow trout (*Oncorhynchus mykiss*)	surfactant	96-h LC50	2.0
	glyphosate	96-h LC50	86
	Roundup	96-h LC50	8.3
Fathead minnow (*Pimephales promelas*)			
	surfactant	96-h LC50	1
	glyphosate	96-h LC50	97
	Roundup	96-h LC50	9.4
Channel catfish (*Ictalurus punctatus*)			
	surfactant	96-h LC50	13
	glyphosate	96-h LC50	130
	Roundup	96-h LC50	16
Bluegill sunfish (*Lepomis macrochirus*)			
	surfactant	96-h LC50	2.0
	glyphosate	96-h LC50	120
	Roundup	96-h LC50	5.0

Table 3.6 The effect of water hardness on the acute toxicity of glyphosate, Roundup and its surfactant to salmonid fishes [73].

Water type	**Salmonid species**	**Glyphosate**	**Surfactant (MON 0818)**	**Roundup**
		96-h LC50 (mg/L)		
Soft water				
	coho	36	3.2	27
	chum	22	2.4	19
	chinook	30	2.8	27
	pink	23	2.8	31
	rainbow	22	2.5	15
Hard water				
	coho	174	1.8	13
	chum	148	1.4	11
	chinook	211	1.7	17
	pink	190	1.4	14
	rainbow	197	1.7	14

3.6 CHRONIC TOXICITY

3.6.1 Phytoplankton

Forest stream periphyton were not affected significantly by aerial treatments of 2 kg ai/ha Roundup [93]. Total biomass, chlorophyll-a and ATP levels were similar in treated and upstream sections of the waterway during the 37-d monitoring period.

Austin et al. [94] used experimental streams to investigate the effects of low concentrations of glyphosate (Vision) on periphyton. Concentrations of glyphosate ranged from 1.1 μg/L to 287 μg/L. It did not appear to inhibit biomass accrual nor was it lethal to any of the pre-treatment dominant species. Growth was enhanced above control levels in treated streams which may be attributable to the phosphate constituent of the herbicide. There was a sudden loss of blackfly (*Simulium* sp.) larvae following treatment with glyphosate, which also could have contributed to increases in periphyton due to reduced grazing. It was concluded that the presence of glyphosate at these environmentally realistic concentrations would have little effects on community succession of periphyton.

3.6.2 Terrestrial Plants

Overall plant diversity and species richness, as judged by indices, were unaffected seven years after ground application of Roundup at the maximum rate of 1.7 kg acid equivalent/ha [95]. Roundup significantly reduced *Vaccinium* sp. compared to controls.

Sullivan et al. [96] reported results from field applications of Vision (2.14 kg ai/ha) in a spruce forest conifer release spray program. Herbaceous species were monitored for 5 years after application. The herbicide caused a reduced crown volume index and species richness of shrubs. The two diversity indices (Shannon-Weaver and Simpson's) were reduced in the first year and maintained a consistent difference for at least five years after treatment. The diversity of herbaceous plant species increased similarly in both treated and untreated areas.

3.6.3 Invertebrates and Fish

Although little information is available on the chronic effects of glyphosate herbicides on invertebrates and especially benthos, it is apparent that crustaceans are about two orders of magnitude more sensitive to Roundup (21-d NOEC = 4.9 mg/L) than technical glyphosate (21-d NOEC = 100 mg/L) [3].

The abundance of benthic macro-invertebrates in a stream and in tributary swamps was similar at untreated sites and sites that received 2.2 kg ai/ha of Roundup. However, after periods of rainstorms leading to flooding, the abundance at the treated sites was 40-50% lower [97]. Estuarine macrobenthos were not significantly affected up to 119 d post treatment with 4.7 L Rodeo + 1L/ha X-77 Spreader in a field study [98].

Tate et al. [99] reported results from a three generation study with snails (*Pseudosuccinea columella*) indicating delayed effects. Snails were continuously exposed to technical glyphosate at concentrations of 0.1-10 mg/L. Little effect was observed in first and second generation snails, however, third generation snail embryos exposed to 1.0 mg/L developed much faster than embryos exposed to 0.1 or 10 mg/L. At 0.1 mg/L hatching was slightly inhibited, while at 10 mg/L it was inhibited by 66%. On the other hand, at these concentrations the egg-laying capacity was increased in snails and abnormalities and polyembryony were observed. The authors indicated that sublethal levels of glyphosate causing increases in snail populations could also increase the distribution of the snail parasite *Fasciola hepatica* (liver fluke). In a previous study, these investigators [100] detected sublethal changes in the same species of snail. For example, glutamic/oxaloacetic transaminase (GOT) activity was significantly increased at 0.1 mg/L glyphosate and was also significantly higher than levels resulting from exposure to 1 and 10 mg/L. Glutamic pyruvic transaminase (GPT) activity was decreased significantly at 0.1 mg/L or higher. Protein content in snails was significantly higher at 1.0 mg/L glyphosate.

In a 14-d study, brown garden snails (*Helix aspersa*) were fed 5 g/kg diets glyphosate [101]. Although mortality was observed, weight and size were significantly reduced compared to controls.

Aerial applications of 5.8 L/ha Rodeo + X-77 Spreader + ChemTrol drift retardant to pothole wetlands in North Dakota generally did not adversely affect invertebrate populations [102]. One and two years post treatment, numbers of copepods, ostracods and cladocera species were not different between treated and control wetlands, however, copepods were generally more abundant in the second year after treatment. Oligochaets and crustaceans were also unaffected, whereas gastropods were higher in treated areas. Both predacious and non-predacious species of chironomid larvae were significantly more abundant in treated wetlands, as were predacious hemipterans. Chaoborid larvae were consistently less abundant in treated areas, although direct and indirect responses could not be differentiated.

Coho salmon smolt adaptation to water salinity changes was unaffected by Roundup at concentrations of 2.8 mg/L during a 10-d period [103]. Morgan and Kiceniuk [104] exposed rainbow trout to concentrations of Vision less than what could be expected to occur in the environment following application. Trout were exposed to 6.25, 25 and 100 µg ai/L as Vision in flow-through tanks for a period of 60 d. There were no significant effects on foraging activity, agonistic activity, growth in weight or length, or number of lesions in gills and livers. However, a behavioural response to stimulation, "wig-wags" was significantly altered. After 1 month of exposure to a mean concentrations of 45.75 µg/L, fish performed wig-wags more frequently than controls. After two months, fish exposed to 4.25 µg/L performed significantly fewer wig-wags.

Carp showed a significant increase in liver alkaline phosphatase activity after 14 d of exposure to technical glyphosate at 2.5-10 mg/L. Similar increases in phosphatase levels were seen in heart of fish at 10 mg/L. Increased GOT and GPT activity in liver and kidney was also observed. Gills of fish exposed to 5 mg/L showed epithelial hyperplasia and edema. These observations were more pronounced at 10 mg/L [92]. Rainbow trout showed avoidance of Roundup in streams at concentrations of >40 mg/L [105].

Stressed behaviour was observed in caged coho salmon in an oversprayed tributary (2.0 kg ai/ha Roundup) in the Carnation Creek watershed [106]. For two weeks, trapping catch of juvenile coho per unit effort was smaller in treated streams than controls. These short term toxic impacts were not considered serious enough to restrict the use of Roundup. In cold water streams, of greater concern may be indirect effects such as increased water temperature due to shade plant removal by herbicides, as well as nutrient content of the water and leaf litter input. In a Carnation Creek tributary, daily maximum water temperatures increased for two years post treatment, phosphate concentrations doubled for two years and leaf litter inputs were reduced by 94%, also for two years, [106]. No significant long term impacts were detected on salmon after treatment with Roundup.

3.6.4 Birds and Mammals

Santillo et al. [107] reported that insectivorous shrews and herbivorous voles were less abundant in forest areas treated with Roundup at a rate of 1.7 kg ai/ha. The significant reduction in the number of shrews was maintained for three years after application whereas the population of voles recovered. Omnivorous deer mice were not affected by Roundup application, even at 3 kg ai/ha [108]. Redback vole populations were reduced by 80% in a 1 year study involving the treatment of a cut-over area with 1.1 kg ai/ha Roundup. No treatment related changes were observed in small mammal populations inhabiting treated (2.14 kg ai/ha Vision) forest land over a 5-year period [96].

Results from Oregon field studies indicate that Roundup application (2.1 L/ha) can modify the density and habitat use of birds [109]. Although bird density and diversity were similar in treated and control sites during the three year study, individual species declined on Roundup treated sites. Relative densities of the rufus hummingbird, MacGillivray's and Wilson's warbler as well as of rufus-sided towhee and white-crowned sparrow declined in the first year after treatment. Some species, on the other hand, increased in density on treated sites, these included the willow flycatcher orange-crowned warbler and American goldfinch.

At least a 25% change in density occurred in the above species, but only densities of towhees and goldfinches were significantly changed. Interestingly, all species that declined in numbers during the first year after treatment, increased in density during the second year and all species that increased during the first year decreased by at least 20% during the second year. The use of shrub cover decreased for all bird species but one, while the use of deciduous tree cover generally increased.

The number of American coots were higher in glyphosate treated wetlands (5.8 L/ha Rodeo + surfactant + drift control agent) than in control wetlands, whereas soras were less abundant in treated area during the first year and recovered to pretreatment levels in the second year [110]. Coot numbers were positively correlated with percent coverage of open water, which was greatly increased by glyphosate treatment. Red-winged blackbirds, yellow-headed blackbird and marsh wrens were all less abundant in treated wetlands where cattail densities were drastically reduced. There was a positive correlation between percent coverage of live emergent vegetation and bird abundance [111]. Three species of *Anas* ducks as well as ruddy ducks were unaffected during the first year, whereas their numbers increased relative to control in the second year [112].

Solberg and Higgins [113] also reported increased use of treated wetlands (2.8L/ha Rodeo) by waterfowl. Cattail density was reduced for up to four years.

3.6.5 Reproductive, Immune and Other Responses

The effects of glyphosate on semen characteristics were investigated in rabbits [114]. Mature New Zealand rabbits were studied for an 18-week period; 6 weeks pretreatment, 6 weeks during treatment and 6 weeks posttreatment. Test animals were given low doses (1/100 LD50) and high doses (1/10 LD50) of encapsulated glyphosate once per day. The body weight of rabbits significantly decreased at both dose levels upon treatment. At the low dose, no recovery was seen for this endpoint, however, at the high dose, body weights were significantly higher than pretreatment levels during the recovery period. All the endpoints investigated on semen characteristics were significantly altered by both treatment levels. These included reduction in semen volume, sperm content and fructose content (energy source), and increase in abnormal and dead sperm cells. These changes were similar at both treatment levels. During the recovery period, most of these four variables did not return to pretreatment levels with the exception of the frequency of dead sperm. Incidence of abnormal sperm increased further during this time at both concentrations. The authors speculated as to the mechanism of action of glyphosate induced sperm toxicity, but could not state whether changes were due to direct cytotoxicity or other effects.

In a follow up article, Yousef et al. [115] reported that glyphosate reduced sperm motility *in vitro* with an IC50 of 48.2 µM in human sperm and 23.5 µM with rabbit sperm. Sperm motility decreased in a dose dependent manner, and increased over time.

Flaherty et al. [116] did not observe any effects on natural killer cell or cytotoxic cell function *in vitro* when exposed to glyphosate or Roundup. On the other hand, El-Gendy et al. [117] found evidence of adverse effects on the immune function of tilapia. Fish were exposed to formulated glyphosate (48%) (exact product not stated) for 96 h at 1/1000 field recommended concentration. Cellular immunity was gradually reduced, reaching maximal depression after 4 weeks post treatment. *In vitro*, humoral immune function (plaque forming cell count) was also decreased in a dose dependent manner. A concentration of 10 µM reduced splenocyte viability by 22% and 100 µM reduced plaque forming cell counts by 76.2%. The ED50 for the latter was 1.65×10^{-2} M. Serum antibody titres decreased in fish over time.

CD-1 mice showed no effects on survival in a 13-week feeding study at glyphosate concentrations of 5 g, 10 g, and 50 g/kg diet. Liver weight was increased at 10 g/kg; growth reductions as well as increased brain, heart and kidney weights were observed at the highest treatment level. The NOEL was calculated to be 10 g/kg diet (1.89 g/kg bw per day) [118]. However significant increases in liver weight at this level suggest that the 5 g/kg is the more appropriate NOEL.

In more recent studies [119], with F-344 rats and B6C3F mice, reduced weight gain was observed in rats given 25 and 50 g/kg diet, without a change in feed consumption. Liver, kidney and testes weights were increased. Alkaline phosphatase and alanine amino transferase activity was increased at >6250 mg/kg diet. Lesions were observed in rat salivary glands and parotid glands, the incidence of which was dose dependent. Salivary gland lesions were observed at the lowest test concentration of 3125 mg/kg diet. In mice, lesions in the parotid glands were seen at 6250 mg/kg but not in salivary glands. These lesions could also be induced by subcutaneous doses of a β-adrenergic agonist, indicating that glyphosate may be acting as a weak adrenergic agonist [119].

3.7 POTENTIAL FOR GLYPHOSATE HERBICIDES TO ACT AS ENDOCRINE DISRUPTORS

Our working definition of an endocrine disrupting compound:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

Although the literature does not provide clear evidence of endocrine disruption, there are studies indicating impacts on immune function in fish [117] and reduced sperm content and viability in rabbits [114,115]; in neither study however, could the mechanisms responsible for these effects be clearly linked to endocrine system modulation. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects. Clearly, the lack of information currently available on the sublethal effects of glyphosate herbicides indicates the need for further research.

Some glyphosate formulations and tank mixtures contain surfactants (nonylphenoxy polyethoxylates) which are microbially degraded in the environment to nonylphenol, a potent estrogenic compound [34,120].

3.8 RISKS TO ONTARIO ENVIRONMENTS AND RECOMMENDATIONS

It is extremely difficult to estimate environmental risk presented by glyphosate, due to the lack of environmental concentration data. Very little surface water residue data is available for Ontario, where glyphosate is now the second most widely used agricultural pesticide. In addition, non-agricultural uses of this herbicide may be as much as 70-100% of the agricultural use volume. Diverse use patterns likely result in a wide distribution of glyphosate, increasing the risk of its entry into urban and agricultural runoff and ultimately, aquatic ecosystems. Little of environmental concentration data that is available has been obtained from routine sampling, but from field studies of fate and effects carried out in forestry operations with aerial application; not applicable for agricultural or urban use scenarios.

The Canadian Water Quality Guideline for the protection of aquatic life of 65 μg/L (Roundup) may well be protective for aquatic species, given the lowest LC50 value available is 1000 μg/L (algae). However, sediment may be the more relevant environmental compartment of exposure, given the chemical characteristics of the compound. Glyphosate residues often increase in sediment during a growing season, sometimes reaching concentrations of about 1 μg/g, and may persist for several months [23,25,33,34]. The availability of glyphosate to sediment-dwelling organisms is not well understood; there is a critical lack of sediment residue data as well as toxicity data for benthic organisms. Although most toxic effects in non-plant species appear to be caused by surfactants, there is no data on the environmental concentration and fate of these chemicals.

There is limited evidence that sublethal responses may occur at environmentally relevant concentrations. For example, snail development and enzyme activity was altered at 100 μg/L and trout behaviour is influenced at levels as low as 6-10 μg/L [99,100,104]. The ecological implications of such subtle effects are essentially unknown especially in the context of all the variables and stressors acting on populations.

Of even greater significance may be the alteration of species composition and diversity in streams due to reductions in or removal of riparian vegetation [94-96]. Water temperature increases could inhibit fish spawning and hatching and reduced leaf litter inputs will adversely impact shredder and grazer invertebrates and thus energy flow in streams.

The endocrine effects of glyphosate and surfactants used in formulations have yet to be evaluated thoroughly. Although it may be unlikely that estrogenic effects could occur from exposure to the active ingredient, some non-ionic surfactants have been known to cause this response. The following issues should be priorities for further evaluation of the extent and degree of risk posed by glyphosate herbicides:

- a more comprehensive monitoring program of surface water and sediment concentrations of glyphosate should be implemented in agricultural and urban areas with relevant detection limits. There is a particular need for sediment data given the chemical attributes of the compound.
- surfactants used in glyphosate formulations need to be evaluated for environmental fate and effects.
- surfactants containing polyethoxylates should be evaluated for endocrine disruption potential.
- critical life stages of sensitive aquatic species should be used in evaluating toxicity of glyphosate and its surfactants.
- few studies have actually assessed endpoints associated with endocrine disruption; more research is needed to provide this information.
- the availability of glyphosate to benthic invertebrates and its effects on these organisms should be studied due to its accumulation and long half life in sediments.
- glyphosate is applied in mixtures in the field and assessment of possible synergistic effects should be conducted.

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4.0 METOLACHLOR

4.1 DESCRIPTION AND USE

Metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide) is a commercial class herbicide in the chloroacetanilide family used for annual grass control in field corn, soybeans, potatoes, cole crops, and beans, amongst others. It is also registered for use on several fruit varieties, ornamental plants, and woodland applications. In Ontario, metolachlor is registered for use on food/feed crops and outdoor ornamental plants. In the U.S. it is registered for a number of other uses including turf, rights of way and commercial and industrial applications. Metolachlor was first marketed in Canada in 1977 by Ciba-Geigy and is currently registered by Syngenta Crop Protection Canada Inc. The herbicide is a germination inhibitor and is applied preemergence, preplant incorporated or early postemergence mainly for the control of grassy weeds at rates of 1-4 kg ai/ha. In corn, the incorporation depth is about 10 cm. Weeds controlled include crabgrass, goose grass, fall panicum, pigweed, foxtails, and yellow nutsedge [1,2].

In Ontario, metolachlor is marketed under several trade names containing different formulations. Metolachlor is often tank mixed with fertilizers, crop oil, non-ionic surfactants, and other herbicides including atrazine, Bladex and Roundup in corn production and Senocor, Lexone, Pursuit, Lorox, and Afolan in soybean production (Table 4.2) [2]. To reduce leaching to groundwater and prevent carryover to the following growing season, it is advised that metolachlor be applied to soils containing between 1 and 10% organic matter [2]. Ground application has been the method of choice for all use sites, although some formulations may be applied through irrigation systems.

Historically, metolachlor has been a racemic (50:50) mixture of two stereoisomers, of the *S* and *R* optical isomers. More recent production (since 1996), however, involves the enrichment of the technical material with the S-enantiomer (S-metolachlor), resulting in a *S*/*R* ratio of 88:11 [3]. These newer formulations have been marketed in the U.S. since 1996 and in Canada since 1998. The S-metolachlor isomer has a higher herbicidal potency compared to the *R*-isomer; about 95% of the herbicidal activity is accredited to the two *S* stereoisomers. Consequently, the same biological effects may be obtained with about 35% less environmental load.

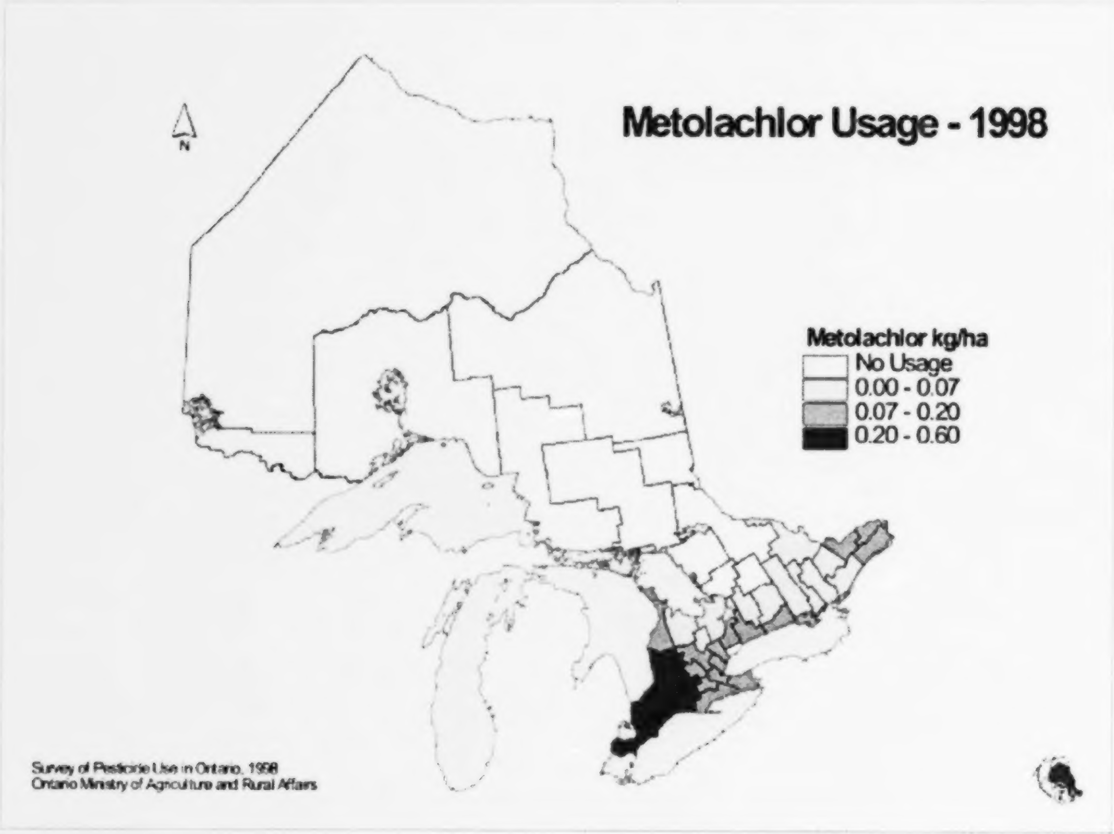
Metolachlor is the most heavily used pesticide in Ontario and its use rate has remained relatively steady since it took the place of alachlor, a closely-related chloroacetanilide herbicide which was removed from the market in 1985 due to concerns about carcinogenicity. The use of metolachlor in Ontario agriculture more than doubled between 1983 and 1988 (842 tonnes versus 1724 tonnes [4]). An estimated 1376.6 tonnes of metolachlor were used in Ontario during the 1998 growing season (Fig. 4.1). Of this amount, 773 tonnes were used in corn production, 553 tonnes in soybeans, 6.4 tonnes in dry beans and 3.2 tonnes in winter wheat. Metolachlor use accounts for one third of the total herbicide use in Ontario. In the U.S., about 27 000 tonnes of metolachlor are used in agriculture annually (72% in corn, 17% in soybean and 5.3% in sorghum).

The U.S. EPA issued a Reregistration Eligibility Decision (RED) for metolachlor in 1995 [5] which indicated that several data gaps exist in the environmental fate and effects database of this pesticide. Specifically, phytotoxicity, aquatic toxicity, avian reproduction, and groundwater contamination were of concern. Data call-in notices were issued by the EPA in 1993 and 1994. In the U.S., metolachlor is subject to the proposed Pesticides and Groundwater State Management Plan for use within a state. In 1996, a lifetime health advisory of 70 μg/L was established for potable water. As stated by the RED for metolachlor, the EPA determined that all uses of metolachlor with the exception of soybeans, potatoes and peanuts as currently registered will not cause unreasonable risk to humans or the environment. An RED for use on soybeans, potatoes and peanuts could not be issued as metolachlor accumulates in these foods and exceeds maximum residue levels (MRLs) even after processing. The EPA classified this herbicide as a Group C, or possible human carcinogen. An acceptable daily intake, or reference dose, of 0.1 mg/kg body weight was established using an uncertainty factor of 100. At the time of the release of the RED, the EPA was evaluating legal challenges related to the coordination of actions under the Federal Food Drug and Cosmetics Act section 409's Delaney Clause. This clause provides that a food additive regulation for a processed food may not be established for a pesticide that induces cancer in humans or animals.

Table 4.1 Physical and chemical properties of metolachlor.

CAS number	51218-45-2
Chemical formula	$C_{15}H_{22}ClNO_2$
Molecular weight	283.8
Specific gravity	1.08 @ 20°C
Vapour pressure	1.3×10^{-5} @ 20°C
Henry's Law constant	3.7×10^{-7}
Solubility in water	530 mg/L @ 20°C
Log K_{ow}	3.13
K_{oc}	0.2-0.47 m^3kg^{-1}
K_d	0.76×10^{-3} to 1.75×10^{-3} m^3kg^{-1}

Figure 4.1 Metolachlor use in the Great Lakes Basin.


Metolachlor Usage - 1998
N
Metolachlor kg/ha
No Usage
0.00 - 0.07
0.07 - 0.20
0.20 - 0.60

Survey of Pesticide Use in Ontario, 1998
Ontario Ministry of Agriculture and Rural Affairs

Table 4.2 Commercial formulations containing metolachlor registered for use in Ontario

Herbicide product	Concentration (g ai/L)
Broadstrike Dual (metolachlor/flumetsulam)	923
Dual (metolachlor)	960
Dual II (metolachlor/benoxacor; a safener which enhances the metabolism of metolachlor in crops)	935
Dual II Magnum (*S*-metolachlor/benoxacor)	915
Dual Magnum (*S*-metolachlor)	915
Primextra Light (metolachlor/atrazine; 2:1)	500
Primextra II Magnum (*S*-metolachlor/benoxacor/atrazine)	720
Check Mate (metolachlor/linuron)	-

Table 4.3 Selected recommended uses of metolachlor in Ontario agriculture [2].

Crop Protected	Formulation	Application rate (per ha)
Corn (field and sweet)	Dual II Magnum +	1.25-1.75 L
	Roundup +	2.3-2.5 L
	Aatrex Nine-O or	1.1-1.7 kg
	Primextra II Magnum	3-4 L
	Dual +	2.0-2.7 L
	Touchdown 480	2.63-2.5 L
	Dual II Magnum +	1.2-1.72 L
	Aatrex Nine-O	1.1-1.7 L
Soybeans	Broadstrike Dual +	2.4 L
	Roundup +	2.5 L
	Touchdown 480	2.5 L
	Dual +	1.7-2.7 L
	Touchdown 480	2.3-2.5 L
	Dual +	1.7-2.7 L
	Lexone DF or Lexone + Sencor 75 DF	0.2-1.5 kg
	Dual Magnum +	1.1-1.75 L
	Pursuit (imazethapyr)	0.3-0.42 L
	Dual Magnum +	1.1-1.7 L
	Lorox DF or Afolan/linuron	1.7-2.3 kg

4.2 ENVIRONMENTAL FATE AND CONCENTRATIONS

4.2.1 Soil

Metolachlor is moderately to highly persistent in soils with an aerobic soil DT50 of 7 to 292 days. It is mobile to highly mobile in soils, and is often detected in runoff water and groundwater in high use areas. Degradation is mainly through microbial metabolism. Volatilization from soil is not thought to be extensive. Metolachlor is slightly soluble in water and is moderately well sorbed by soils. Extensive leaching is reported to occur in soils of low organic matter content [1].

As summarized by the U.S. RED on metolachlor [5] it has a soil photolysis half-life of 8 d under natural sunlight. The DT50 in a sandy loam soil under aerobic conditions was 67 d, while under anaerobic conditions the DT50 was 81 d. It was found to be highly mobile in a sandy soil ($K_d = 0.08$) and moderately mobile in a sandy loam soil ($K_d = 4.81$) based on soil column leaching. Laboratory studies indicated that volatilization from soil is not a significant mode of dissipation (0.05% of applied dose volatilized per day).

Peter and Weber [6] found that adsorption of metolachlor to soil positively correlated with the organic matter and clay content of the soil. Humic substances are the most important organic components influencing soil absorption. Adsorption of metolachlor is thought to be due to hydrogen bonding between the pesticide molecule and carboxyl and hydroxyl groups of humic substances [7]. In an Ontario field study conducted with a sand soil (91.5% sand) with low organic matter (0.7%) metolachlor exhibited limited downward movement (to only 10 cm) after 386 mm of rainfall [8]. Metolachlor leached to a depth of 30 cm in a field study conducted near Ottawa at very low concentrations; however, it occurred in tile drainage water 0.6-0.9 m below the surface, at concentrations of up to 12 µg/L [9]. Metolachlor was transported down the soil column via macropore transfer with water.

Degradation of metolachlor followed first order kinetics with a DT50 of 72 d after preplant incorporation in the top 10 cm layer of a clay loam soil. This value for preemergence application was 39 d. Chesters et al. [10] reported that 4.5% of the applied amount was lost in surface runoff from a field treated with 1.1 kg ai/ha. This loss was after 12 cm of rain over 7 d on an 8% slope. The primary factor affecting the fate of metolachlor in soil is biodegradation. Aerobic soil microbial activity resulted in 4.8% of the ^{14}C-labelled herbicide being converted to $^{14}CO^2$ [11]. Less than 8% of the 5 mg/kg parent compound remained in a clay loam soil after 84 d. In a sterilized soil sample, 65% of the original amount remained after the same time period. Although microbial degradation removes the parent compound from soil, complete mineralization does not occur. In microbial screening studies, Saxena et al. [12] were unable to isolate microbes capable of mineralizing metolachlor. Some *Bacillus* and *Fusarium* species were able to transform the herbicide to some extent.

In a 3-year field experiment, Frank et al. [13] determined a soil DT50 of 80-142 d for this herbicide. Treatment consisted of a single preplant/preemergence application of 2.4-2.6 kg ai/ha (in the Dual EC formulation) on a clay loam soil (60% sand, 27-31% silt and 2-3.5% organic matter) cropped with corn. Metolachlor residues were not detected below 30 cm in the soil, but were detected in the tile drainage. Less than 0.01% of the total amount applied reached the tiles. Groundwater also contained metolachlor at a depth of at least 4.6 m at concentrations of 0.56 µg/L. Residues in the soil persisted to the next growing season. Hall et al. [14] examined the effects of various formulations and application rates on the leaching potential of metolachlor. Microencapsulation (ME) and adjuvant-amendment (AA) reduced the mobility of the herbicide from 2.3% loss (commercial formulation) to 0.8% loss (ME) and 0.5% loss (AA). Eleven percent of the applied amount of metolachlor moved through a 30 cm soil column in 3.5 L of water over a period of 24 d.

The loss of metolachlor from a clay loam field treated at 1.1 kg ai/ha and receiving 3.8 cm of simulated rain amounted to 4.5% in surface runoff [15]. By comparison, 10% was lost in runoff from small plots during large simulated rainfalls [10].

4.2.2 Water and Sediment

Metolachlor is among the five most commonly detected herbicides in water and has been detected in surface and groundwater in several states and in Ontario [1, 10]. During the early 1980s in southwestern Ontario, mean metolachlor concentrations in the Grand, Saugeen and Thames Rivers were 0.9, 0.7 and 3.6 µg/L, respectively [16]. Raw water at Ontario municipal waterworks contained metolachlor residues at concentrations of up to 5.1 µg/L during 1985 [17]. In 1986, 25 municipal waterworks were monitored for pesticide residues in Ontario. Metolachlor was found in treated and untreated water at concentrations of up to 5.97 and 15.0 µg/L, respectively. During 1987, 7 of 12 samples from the Sydenham River contained metolachlor with a maximum level of 14 µg/L and 6 of 12 drinking water samples contained up to 16 µg/L [18]. Although some of these concentrations exceeded the Canadian Water Quality Guidelines for the protection of aquatic life of 7.8 µg/L [19], they did not approach the Canadian Water Quality drinking water guideline of 50 µg/L [20].

In a study of herbicide runoff in paired agricultural watersheds of the Thames River in southern Ontario, Struger et al. [21] sampled extensively over a 4-year period (1988-1991). Samples were collected during base flow periods (grab samples), high flow periods (8 samples over 12 h during storm events) and on a continuous basis (weekly composite flow-proportional samples). Metolachlor concentrations in 129 grab samples did not exceed the CCME [19] Water Quality Guideline for the protection of aquatic life of 7.8 µg/L. Of 286 storm event sampling sessions, 8 (2.8%) samples exceeded the guideline. Of 185 weekly continuous samples, none exceeded this guideline. The maximum concentration of metolachlor was 34 µg/L.

The concentrations of metolachlor in the Payne River in eastern Ontario was determined during the 1991-92 field seasons, with samples collected weekly from April to October each year [25]. Metolachlor was found infrequently, with only two detections in 1991 and three in 1992. A total of 0.01-0.03% of the amount applied (2.22 kg ai/ha) was estimated to reach the river. Metolachlor was not detected in tile drainage; however, samples were not taken during or after storm events. Residues were also detected in river water during dry conditions, indicating that groundwater discharge is an additional source of surface water contamination. The maximum concentration in river water was 0.43 µg/L in May.

Buttle [35] reported metolachlor loss in surface runoff from a southern Ontario cornfield treated with 2.64 kg ai/ha metolachlor. Runoff contained up to 293 µg/L metolachlor in water and 1640 µg/kg in sediment. Bishop et al. [36] reported 0.22-1.22 µg/L of metolachlor in samples from the Holland river watershed during 1991-93.

In a survey of herbicides in Ontario surface waters (1986-1990) metolachlor was the second most commonly detected compound, having measurable residues in 6% of samples [13]. Atrazine was the most commonly detected herbicide with detections in 72% of samples. Similar finding were reported by Maguire and Tkacz [26] in samples were taken from the mouths of the Yamaska River in Quebec and five of its tributaries, during the summers of 1986 and 1987. Metolachlor and atrazine were most frequently found. A high concentration of 4.66 µg/L was recorded for metolachlor in July of 1987. In a more recent survey of the Canadian tributaries of Lakes Erie and Ontario between 1998 and 2000, Struger et al. [22,23] found metolachlor in 10 or 127 Lake Erie samples and 5 of 50 Lake Ontario samples. Maximum concentrations were 22 and 0.51 µg/L for the two lakes, respectively.

In the U.S., metolachlor was detected in 82% of nearly 2000 surface water samples with maximum concentrations of 138 µg/L. The 85th percentile of the concentration distribution was 11.5 µg/L. In Lake Erie tributaries, peak metolachlor concentrations reached 97 µg/L as reported by Richards and Baker [24]. Jaynes et al. [32] found metolachlor and atrazine residues in about 50% of all surface water sample collected from an agricultural drainage basin. Maximum metolachlor concentration reached 33 µg/L, but median levels were below 1.0 µg/L. Annual losses from the watershed ranged from 0.05%-1.6% of the total herbicide applied.

In a recently published survey of pesticides in a tributary of Chesapeake Bay, Harman-Fetcho et al. [28]

report that although metolachlor and atrazine were used to a similar extent (equal volume) maximum concentrations of metolachlor were 25-40 times lower than that of atrazine (70 versus 3000 µg/L, respectively). This observation is consistent with results of other surveys, even though the water solubility of metolachlor is greater than that of atrazine (530 mg/L versus 33 mg/L). The authors stated that metolachlor is less mobile in soil and is transformed faster than atrazine in the environment.

In the U.S., metolachlor was detected in groundwater with a frequency of 14.6% (1034 sites sampled) at a maximum concentration of 5.4 µg/L [27]. Chesters et al. [10] reported that up to 12 µg/L was detected in groundwater in agricultural areas of Wisconsin. Groundwater concentrations of frequently detected pesticides in Iowa were evaluated in a survey spanning 13 years (1982-1995) [34]. The frequency of detections and median concentrations of metolachlor increased during the period. Of the 1019 wells sampled for the survey, none contained detectable residues of metolachlor during 1982-86; 4.8% were positive during 1987-91; and 14.1% were positive during 1992-95. Most of these detections occurred at intermediate well depths (16-30 m). Metolachlor use increased by more than 50% between 1982-95 in Iowa. The contamination of groundwater by metolachlor and atrazine was compared in conventional tillage (CT) and no-till (NT) operations [33]. Metolachlor was detected in only 25% of the samples, while atrazine and its metabolite deethyl atrazine were almost always detected. There was not tillage treatment effect on metolachlor leaching. The maximal concentration of metolachlor measure was 15 µg/L under CT and 10 µg/L under NT.

Several recent investigations into the fate of metolachlor and other chloroacetanilide herbicides have indicated that the metabolites of these chemicals are found at much higher concentration in water than their parent compounds [29,30,31]. Furthermore, two major metabolites of metolachlor, metolachlor ethane sulfonic acid (ESA) and metolachlor oxanilic acid (OA) can persist in soils for four years or more after application. The transformation of the parent compound to metolachlor ESA is a biologically mediated process, which occurs through glutathione conjugation in microorganisms, plants and animals [29]. Due to the very low vapour pressures of these metabolites, they have not been extensively analysed for in environmental samples until the late 1990s. Both ESA and the OA metabolites are much more soluble in water that metolachlor. Phillips et al. [29] sampled tile drainage as well as surface water for metolachlor and the ESA and OA metabolites; concentrations of metabolites were always higher than those of the parent molecule and were consistently detected in drainage water. ESA concentrations reached 5-20 µg/L in tile drainage water; OA concentrations ranged from 1-10 µg/L; metolachlor concentrations ranged from 0.003-0.1 µg/L. In receiving stream water, metabolite concentrations ranged from 0.2-0.5 µg/L compared to metolachlor concentrations of 0.006-0.3 µg/L. The persistence of the metabolites in soil was brought to light when samples taken from tile drains before application of metolachlor in 1997 contained ESA and OA concentrations exceeding 10 and 3.1 µg/L; metolachlor was 0.02 µg/L. Metolachlor had last been applied during the 1993 growing season, indicating persistence over a 4-year period.

Metolachlor is not susceptible to hydrolysis. Aqueous hydrolysis was slow at a pH range of 1-9, having a half life of > 200 d (20°C) [37]. Similarly, aqueous photolysis is an insignificant environmental fate pathway for metolachlor. Kochani and Maguire [38] determined that the aquatic photolysis half life in near surface lake water was 22 d in the summer and 205 d in the winter at 40°N latitude. In natural sunlight, only 6% photodegradation was observed over a 1-month period. Liu et al. [39] found that metolachlor was very stable in natural lake waters. No apparent biotransformation occurred in three Ontario lake waters over a period of 170 d. Native microorganisms that were able to biodegrade PAHs in water were not able to do so with metolachlor.

In an aquatic field mesocosm study Graham et al. [40] studied the fate of metolachlor and alachlor. Metolachlor had DT50 of 33-46 d. The ethane sulfonic acid (ESA) metabolite of metolachlor reached a concentration of 0.5 µg/L in water.

The microbial degradation of metolachlor was extensively reviewed by Chesters et al. [10]. In anaerobic sediment, the half life of the compound was 26-42 d [41]. After 56 d, 41% of the ^{14}C label was extracted from the non-sterile compartment, the remainder was biologically bound or strongly sorbed to sediment. 79% of the ^{14}C label was extracted from the sterile sediment. Most of the degradation was found to be biological (58%) rather than chemical (8%).

4.2.3 Air

Metolachlor has been detected in rainwater at a concentration as high as 2.4 µg/L, coinciding with agricultural use [42]. Volatilization rates of 30-90 g/ha/d were reported for three soil types treated with 80 µg/L metolachlor at 35°C. Volatilization was increased in soils with low organic matter content; increased temperature (45°C) caused a 4-fold increase in volatilization losses and lower temperatures (25°C) resulted in a 4-fold decrease in losses. The loss from plant surfaces may be significantly greater than from soil. Close to 50% of metolachlor volatilized from a glass surface whereas estimated loss via this route was 11-37% from plant surfaces. Zhu et al. [43] detected metolachlor in air at a concentration of 9.8 ng/m^3 in southern Ontario. The samples were collected by aircraft flying over agricultural and forest land from Ottawa to southern Ontario at an altitude of 40-160 m.

Rice and Chernyak [44] detected metolachlor in arctic fog at a concentration of up to 2 ng/L. Samples were collected over the Bering and Chukchi Seas and the Pacific Ocean. The water portion of fog was found to concentrate several semi-volatile pesticides. The vapour fraction of the samples contained no detectable residues of metolachlor. Prueger et al. [45] reported that metolachlor loss due to volatilization from treated corn fields was as high as 22% of the applied amount. The Dual formulation was applied at a rate of 2.24 kg ai/ha using either broadcast treatment or banded treatment. Airborne metolachlor residues were captured in foam plugs placed at various heights above the soil which were monitored for 10 d. Broadcast application of Dual resulted in a 3-fold higher volatilization loss (22%) compared to banded treatment (6%). About 95% of the material was lost within 12 hours of treatment. Maximum aerial concentrations were measured at 2.5 m and at a concentration of 11800 ng/m^3.

4.3 Bioconcentration and Metabolism

4.3.1 Plants

Plants that metabolize metolachlor retain the metabolites for some time. Metolachlor is absorbed and translocated in both susceptible and tolerant plants, however, tolerant plants rapidly deactivate it via conjugation with glutathione. Chesters et al. [10] describe the biochemical pathway of metolachlor metabolism in plants.

4.3.2 Mammals

Animals generally eliminate metolachlor and its metabolites quickly. Metolachlor is absorbed and excreted in urine and faeces of rats, goats and poultry. No parent compound was detected in excreta or tissues in these test animals [46]. In rats and poultry, trace amounts of metabolites were found in kidney, liver and blood. The *in vivo* half life in male rats treated with 31 mg/kg body weight was 28 h. LeBaron et al. [37] proposed the major route of metabolism involves oxidation to the carboxylic acid while the pathway involving glutathione conjugation occurs to a limited extent.

4.3.3 Aquatic organisms

Fish readily absorb and eliminate chloroacetanilide herbicides. After a 70-d exposure period to 1.2 mg/L metolachlor, bluegill sunfish (for Latin names for vertebrate species see Appendix 3) showed residue levels of 18 mg/kg in edible tissues and 486 mg/kg in non-edible tissues. Depuration for 28 d decreased the concentrations to

12 and 13 mg/kg, respectively [47]. Bioconcentration factors (BCF) of 6.5-9.0 were reported in catfish edible tissues when exposed to 0.08 mg/L for 30 d [48]. The viscera of the fish had BCFs of 55-92. After 14 d of depuration, concentrations in edible tissues decreased from 0.72 to 0.03 mg/kg.

The green alga *Scenedasmus acutus* had metolachlor concentrations of 10.4 mg/kg after a 1.5-h exposure to 0.1 mg/L. A 2-h depuration period reduced this level to 2 mg/kg [49]. Cruz et al. [50] exposed bluegill sunfish to ^{14}C-metolachlor for 34 in a flow through system. At the end of the exposure period, ^{14}C residues were 13.9 and 220-250 mg/kg in the edible and non-edible tissues, respectively. The authors conducted a detailed analysis of the metabolites extracted from bluegill tissues and proposed a metabolic pathway involving O-demethylation, reductive dechlorination and subsequent glutathione conjugation.

4.4 Toxic Mechanism of Action

The site of uptake is in roots and shoots of seedlings. Chloroacetanilide herbicides are applied prior to plant emergence so that they can be absorbed through roots and or shoots just above the seeds. These herbicides are in general growth inhibitors affecting root elongation. Seedling growth is restricted due to inhibition of cell division [10,51]. The following processes have been found to be affected by these herbicides: protein and lipid synthesis, membrane events, gibberellin-induced responses, respiration and photosynthesis and terpenoid synthesis [1,51,52]. The specific mode of action of metolachlor is not known.

4.5 Toxicity to Non-target Organisms

4.5.1 Phytoplankton and Macrophytes

The Canadian Water Quality Guideline for the Protection of Aquatic Organisms published in 1991[1] states that "there are currently no acceptable data regarding metolachlor toxicity to algae and macrophytes". Ellgehausen et al. [49] determined a NOEC of 0.1 mg/L for the alga *Scenedasmus acutus*, however, details regarding the study were not provided. In the U.S. RED on metolachlor [5], it is stated that non-target plant studies from the registrant were not available but that a Data Call-In Notice requested this data (seedling emergence test, vegetative vigour test and aquatic plant growth) as of December 1993. St. Laurent et al. [53] compared the toxicities of 9 herbicides to the green alga *Selenastrum capricornutum* using micro plate and flask bioassay procedures. The 96-h EC50 for metolachlor (for cell growth inhibition) was determined to be 50.9 μg/L using the microplate assay and 55.5 μg/L using the flask assay. Day [54] found that concentrations of 274 μg/L metolachlor reduced respiration rates in a periphytic community composed of diatoms, green algae, cyanobacteria and protozoans in experimental streams.

Day and Hodge [55] evaluated the effects of metolachlor, five transformation products and the commercial safener benoxacor in two species of algae as well as the floating macrophyte duckweed. The endpoint measured was inhibition of growth or decrease in cell biomass for the algae and decrease in frond production and dry weight in duckweed. The green alga *S. capricornutum* was most sensitive to metolachlor with a 72-h EC50 value of 37.2-55.8 μg/L, as determined in the microplate assay. Duckweed was less sensitive having a 7-d EC50 of 304-788 μg/L for frond counts and 766 μg/L for dry weight. None of the five transformation products showed toxic effects on the three species tested at concentrations up to 10 mg/L. The cyanobacterium *Anabaena cylindrica* was not inhibited by the parent compound at the highest concentration of 4 mg/L. Benoxacor caused some growth inhibition in *S. capricornutum* at a concentration of 1.7-2.0 mg/L. These results corroborate those of St. Laurent et al. [53]. As noted by the authors, the transformation products are without chlorine atoms, an attribute which likely contributes to the diminished activity against plants.

Fairchild et al. [56] reported the effects of metolachlor on six species of algae and five species of macrophytes. *S. capricornutum* was again the most sensitive species overall, having a 96-h ED50 of 84 µg/L for cell counts (Table 3.4). The macrophyte *Ceratophyllum* sp. was slightly more sensitive with a 14-d EC50 of 70 µg/L. Goncz and Sencic [57] determined the subchronic toxicity of metolachlor on the free-floating fern *Salvinia natans*. The 28-d EC50 values were: chlorophyll-*b* content (50 µg/L); stem length (50 µg/L); number of leaves (75 µg/L); chlorophyll-*a* (80 µg/L); wet weight (150 µg/L).

4.5.2 Aquatic Invertebrates

It has been noted that there are very little data on the toxicity of metolachlor [58]. Kent et al. [1] reported metolachlor toxicity data for only two aquatic invertebrate species, *Daphnia magna* and the midge larva *Chironomus plumosus*. The 48-h EC50 and LC50 for *Daphnia* were 23.5 and 25.1 mg/L, respectively [59,60]. The 48-h LC50 for the midge *C. plumosus* was 3.8 mg/L. The 48-h LC50 for *D. magna* was determined in soft and hard water in eutrophic, mesotrophic and oligotrophic lake waters. The EC50 range was 4.2-7.9 mg/L for soft water and 15.7-16.5 mg/L in hard water. Chronic toxicity was also evaluated over 7 d. The 7-d EC50 for *D. magna* reproduction was 1.4 mg/L in soft water and 11.4 mg/L in hard water (soft water: 4 mg CaCO3/L, hard water: 170 mg CaCO3/L). The green alga *Chlorella* species, used as a food source in the chronic study, exhibited significant mortality at a concentration of 0.5-1.0 mg/L and may have cause indirect effects on the daphnids through food reduction. Fairchild et al. [56] determined a daphnid 96-h EC50 of 0.203 mg/L.

4.5.3 Fish

The U.S. RED for metolachlor lists this herbicide as moderately toxic to freshwater fish, with acute LC50s ranging from 3.9 (rainbow trout) to 10 mg/L (bluegill sunfish). The review by Kent et al. [1] listed data for only six species of fish, with fathead minnows and rainbow trout having 96-h LC50s of 2-11 mg/L. The remaining test species, (guppy, bluegill, catfish and carp) had LC50s of 4.9-15 mg/L. Only one sub-chronic study was found for the current review; it was conducted by the registrant of metolachlor. Fathead minnows were exposed for over 4 weeks to technical grade metolachlor. The NOEC for reproduction was 780 µg/L [61].

4.5.4 Laboratory Mammals and Birds

Metolachlor has a low level of acute toxicity in laboratory mammals. Technical grade material had an LD50 of 2780 mg/kg in rats and a dermal LD50 of >10 g/kg in rabbits. In a subchronic feeding study beagle dogs were fed doses of 500 and 1000 ppm in food for 3 months. The NOEL was >1000 ppm (25 mg/kg/d) [5]. No treatment related carcinogenic effects were observed in a two year feeding study in mice or rats at concentrations of up to 3000 ppm. Another two year study reported a significant increase in neoplastic nodules in livers of female rats at a dose of 3000 ppm (150 mg/kg/d) [5]. Metolachlor was not mutagenic in several assays, including Salmonella assay, chromosome aberration and micronucleus. A two generation reproduction study in rats with doses of up to 1000 ppm in diet revealed a NOEC of 300 ppm based on reduced pup weights [5].

The acute oral LD50 for the mallard duck was >2500 mg/kg [62]; however, mallards and northern bobwhites fed 10 mg/kg metolachlor for 16-17 weeks experienced significant reproductive impairments [cited in 10].

Table 4.4 Toxicity of metolachlor to aquatic organisms. Species indigenous to Ontario are identified by an (I) after the species name.

Species	Common name	Formulation	Dose Method	Time	LC50 or EC50 (mg/L)	Reference
FISH						
Pimephales promelas (I)	Fathead minnow	technical (95.4%)	-[a]	96-h	8	[60]
Pimephales promelas (I)	Fathead minnow	87% EC	-	96-h	8.4	[60]
Pimephales promelas (I)	Fathead minnow	technical (97.4%)	-	>4 weeks	NOEC, 0.78	[61]
Pimephales promelas (I)	Fathead minnow	technical (97.4%)	static	96-h	11.0	[61]
Pimephales promelas (I)	Fathead minnow	technical (97.4%)	flow through	96-h	9.2	[61]
Lebistes reticulata	Guppy		-	96-h	8.6	cited in [1]
Lepomis macrochyrus (I)	Bluegill		-	96-h	10	cited in [1]
Ictalurus punctatus (I)	Channel catfish	technical	-	96-h	4.9	cited in [1]
Carassius carassius	Carp	technical	-	96-h	4.9	cited in [1]
Oncorhynchus mykiss	Rainbow trout	technical	-	96-h	2.0	cited in [1]

Table 4.4 Continued

INVERTEBRATES

Daphnia magna (I)	water flea	technical (95.4%)	-	48-h	23.5	[60]
Daphnia magna (I)	water flea	87% EC	-	48-h	26.0	[60]
Daphnia magna (I)	water flea	technical	Static	48-h	4.2	[58]
Daphnia magna (I)	water flea	technical	-	7-d	NOEC, 1.4-11.4	[58]
Chironomus plumosus (I)	midge	technical	-	48hr	3.8	[58]
Chironomus plumosus (I)	midge	87% EC	-	48-h	4.4	[58]

[a] information not provided

4.6 POTENTIAL FOR ENDOCRINE DISRUPTION

Our working definition of an endocrine disrupting compound:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

No studies are available in the open literature concerning sublethal physiological responses from exposure to metolachlor such as estrogen receptor affinity, steroid hormone modulation etc. Therefore at the time of this writing it is not possible to state whether or not metolachlor may have properties capable of endocrine effects. Unlike most high volume use pesticides such as atrazine and despite being on the market for over 25 years, metolachlor has been subjected to only a few toxicity studies, none of which have been adequate to assess the physiological effects of this pesticide.

4.7 RISK TO ONTARIO ENVIRONMENTS AND RECOMMENDATIONS

Generally, measured concentrations of metolachlor in Ontario waters have been below 5 µg/L [1,57], although concentrations in water during storm runoff have reached 293 µg/L [35] and Burgoin et al. [63] reported levels of 80 µg/L in creek water. High risk areas of extensive metolachlor use exist in Ontario and, given the relatively long aquatic DT50 of the compound, adverse phytotoxic effects can be expected in surface waters under certain conditions. The CCME [19] Water Quality Guideline of 7.8 µg/L for the protection of aquatic life was based on a chronic study conducted with fathead minnows. The NOEC of 780 µg/L for reproductive effects was divided by a safety factor of 100 due to lack of toxicity data on appropriate aquatic organisms. A revised water quality guideline may be needed based on new information. The following issues should be priorities for further evaluation of the extent and degree of risk posed by the metolachlor:

- more aquatic toxicity data is required in all major taxonomic groups.
- long term chronic studies should be done to assess possible effects of endocrine disruption in invertebrates, fish and amphibians.
- environmental concentrations of metolachlor in water and sediment from high use areas should be monitored.
- given the frequency with which metolachlor can be applied with other herbicides and fertilizers, studies should be undertaken to examine toxicity of relevant mixtures.
- toxicity of metolachlor to non-target endemic species should be examined.

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APPENDIX 1: GLOSSARY OF TERMS

Active ingredient – in a pesticide formulation, the chemical with biocidal properties, intended as the pest–targeting agent.

Acute – having a sudden onset, lasting a short time; of a stimulus, severe enough to induce a response rapidly.

Additivity – referring to the toxicity of a mixture of chemicals, which is approximately equal to a simple summation of known toxicities of individual elements of the mixture.

Antagonism – referring to the toxicity of a mixture of chemicals, which is less than a summation of known toxicities of individual elements of the mixture.

Autocrine – cellular communication in an organism in which cells secrete agents that have specific actions on the secreting cell, alone.

Bioaccumulation – process by which chemicals are taken up by organisms.

Bioaccumulation factor – a value that is the ratio of tissue chemical residue to chemical concentration in an external environmental phase such as food or soil/sediment.

Bioconcentration – process by which there is a net accumulation of chemical from external environments into an organism; uptake > elimination.

Biomagnification – the trophic result of bioaccumulation and bioconcentration processes; in which tissue concentrations of chemicals increase as the chemical passes up through two or more trophic levels in a food chain.

Chronic – involving a stimulus that is lingering or continues for a long time.

Endocrine disrupter – an exogenous agent that interferes with the synthesis, secretion, transport, binding action or elimination of natural hormones in the body.

Endocrine system – the communication system in an organism that involves carrying messenger molecules in the bloodstream to distant target tissues; sister to autocrine and paracrine systems.

Estrogen – a family of female sex steroids responsible for producing estrus and the female secondary sex characteristics and preparing the reproductive system for fertilization and implantation of the ovum.

Estrogenic – a chemical having qualities that allow it to function as estrogen in a body.

Formulation – the commercial form of a pesticide; includes active ingredient as well as inert ingredients (surfactants, process impurities, etc.).

Growth regulators – a chemical/hormone that directly influences metabolism of an organism and regulates growth rate.

Hormone – a chemical compound synthesized and secreted by an endocrine tissue into the bloodstream; influences the activity of a target tissue.

Inert ingredient – those chemical(s) in a pesticide formulation that are thought to have no adverse biocidal properties, intended as solubility agents, etc. or with other proprietary functions.

In vitro – in an artificial environment outside the body; often used to describe toxicity tests performed using isolated cell cultures.

In vivo – within the living organism or tissue.

Mechanism of action – the pathway by which a toxicant produces an effect in an organism.

Neuroendocrine system – a major form of communication within the body, in which nerve cells release messenger molecules (hormones) into the bloodstream for transport to a distant target tissue.

Organochlorine – a family of chemicals that includes all chlorinated hydrocarbons.

Paracrine – cellular communication in an organism in which cells secrete agents that influence neighbouring cells.

Pesticide – a substance used to kill undesirable ...fungi, plants, insects, or other organisms ... [a] generic term ... used to describe fungicides, algicides, herbicides, insecticides, rodenticides and other substances.

Surfactant – a surface–active substance that tends to reduce surface tension; used to describe synthetic and natural detergents added to pesticides to increase the solubility of the active ingredient.

Synergism – referring to the toxicity of a mixture of chemicals, which is greater than a summation of known toxicities of individual elements of the mixture.

Target site – the tissue or cell or receptor that a toxicant acts upon to produce a response in the organism.

Testosterone – a steroid androgen (hormone having masculinizing activity) synthesized by the testicular interstitial cells of the male and responsible for the production and maintenance of male secondary sex characteristics.

Thyroid gland – an endocrine gland responsible for regulation of energy metabolism; the two major thyroid hormones are thyroxine and 3,5,3–triiodothyronine.

Toxicant – an agent capable of producing an adverse response (effect) in a biological system.

Toxicity – the inherent potential of a toxicant to cause adverse effects in a living organism when the organism is exposed to it.

APPENDIX 2: GLOSSARY OF ACRONYMS

ai – Active Ingredient

ACh – Acetylcholine

AChE – Acetylcholinesterase

AhR – Aryl Hydrocarbon Receptor

ATP – Adenosine Triphosphate

AT – Androgen Receptor

BCF – Bioconcentration Factor

CAS – Chemical Abstracts Number

ChE – Cholinesterase

CNS – Central Nervous System

DIDT – 5,6 – dihydro – 3h – imidazo(2,1 – c) – 1,2,4 – dithiazole – 3 – thion

DNA – Deoxyribonucleic Acid

DT50 – Time until 50% disappearance of a compound from a media

EAC – Endocrine Active Chemical

EBDC – Ethylene Bisdithiocarbamate

EBIS – Ethylene Bisisothiocyanate Sulfide

EC – Emulsifiable Concentrate

EC50 – Effective concentration causing a response in 50% of a test population

ED50 – Effective Dose causing a response in 50% of a test population

EDA – Ethylene Diamine

EDC – Endocrine Disrupting Compound

EEC – Expected Environmental Concentration

ER – Estrogen Receptor

ETU – Ethylene Thiourea

EU – Ethylene Urea

FSH – Follicle Stimulating Hormone

GABA – Gamma – amino Butyric Acid

hER – human Estrogen Receptor

K_{ow} – Octanol/Water Partition Coefficient

Concentration (In Water) Causing 50% Mortality

LD50 – Oral dose causing 50% mortality

LH – Luteinizing Hormone

LHRH – Luteinizing Hormone Releasing Hormone

LOEC – Lowest Observable Effect Concentration

MATC – Maximum Allowable Toxicant Concentration

MOE – Ministry of the Environment

NAS – National Academy of Sciences

NOAEC – No Observable Adverse Effect Concentration

NOEC – No Observable Effect Concentration

OMAFRA – Ontario Ministry of Agriculture, Food and Rural Affairs

OP – Organophosphorus Pesticide

PAH – Polycyclic Aromatic Hydrocarbon

PCB – Poly Chlorinated Biphenyl

PETD – Polymeric Ethylenethiuram Disulfide

PR – Progesterone Receptor

TBT – Tributyltin

TRH – Thyroid Releasing Hormone

TSH – Thyroid Stimulating Hormone

U.S. EPA – United States Environmental Protection Agency

WHO – World Health Organization

WP – Wettable Powder

APPENDIX 3: LIST OF COMMON AND LATIN NAMES OF VERTEBRATE SPECIES

Fish:
Black crappie: *Pomoxis nigromaculatus*
Bluegill sunfish: *Lepomis macrochirus*
Brown bullhead: *Ictalurus nebulosis*
Carp, common: *Cyprinus carpio*
Carp, crucian: *Carassius carassius*
Carp, Rohu: *Labeo rohita*
Carp, Hamilton: *Cirrhina mrigala*
Catfish, black: *Ictalurus melas*
Catfish, channel: *Ictalurus punctatus*
Congolli: *Pseudaphritis arvilii*
Fathead minnow: *Pimephales promelas*
Gizzard shad: *Dorosoma cepedianum (Lesueur)*
Golden orfe: *Leuciscus idus*
Guppy: *Lebistes reticulata*
Largemouth bass: *Micropterus salmoides*
Mosquito fish: *Gambusi affinis*
Pumpkinseed: *Lepomis gibbosus*
Salmon, Atlantic: *Salmo salar*
Salmon, chinook: *Oncorhynchus tshawytsha*
Salmon, chum: *Oncorhynchus keta*
Salmon, coho: *Oncorhynchus kisutch*
Salmon, pink: *Oncorhyncus gorbusch*
Sockeye salmon: *Oncorhynchus nerka*
Tilapia: *Tilapia nilotica*
Trout, brook: *Salvelinus fontinalis*
Trout, brown: *Salmo trutta*
Trout, rainbow: *Oncorhynchus mykiss*
Sea lamprey: *Petromyzon marinus*
Striped bass: *Morone saxatilis*
Zebrafish: *Danio (Brachydanio) rerio*

Amphibians:
American toad: *Bufo americanus*
Brown froglet: *Crinia insignifera*
Bullfrog: *Rana catesbeiana*
Common frog: *Rana temporaria*
Common toad: *Bufo bufo*
Leopard frog: *Rana pipiens*
Moaning frog: *Heleioporus eyrie*
Pobblebonk frog: *Lymnodynastes dorsalis*
Tiger salamander: *Ambystoma tigrinum*
Western green tree frog: *Litoria moorei*
Wood frog: *Rana sylvatica*

Reptiles:
American alligator: *Alligator mississippiensis*

Birds:
American coot: *Fulica americana*
American goldfinch: *Carduelis tristis*
Chicken: *Gallus domesticus*
Japanese quail: *Coturnix japonica*
Mallard duck: *Anas platyrhynchos*
MacGillivray's warbler: *Oporornis tolmiei*
Marshwren: *Cistothorus palustris*
Northern bobwhite: *Colinus virginianus*
Orange–crowned warbler: *Vermivora celata*
Red–winged blackbirds: *Agelaius phoeniceus*
Ring–necked pheasant: *Phasianus colchicus*
Ruddy duck: *Oxyura jamaicensis*
Rufus hummingbird: *Selaphorus rufus*
Rufus–sided towhee: *Pipilo erythrophthalmus*
Sora: *Porzna carolina*
White–crowned sparrow: *Zonotrichia leucophrys*
Willow flycatcher: *Empidonax traillii*
Wilson's warbler: *Wilsonia pusilla*
Yellow–headed blackbird: *Xanthocephalus xanthocephalus*
Zebra finch: *Pewphila guttata*

Mammals:
Rodents
House mouse: *Mus musculus*
Laboratory strains:
- B6C3F mice: *Mus musculus*
- CD–1 mice: *Mus musculus*

Deer mice: *Peromyscus maniculatus*
Norway rat: *Rattus norvegicus*
Laboratory strains:
- Fisher 344 rat: *Rattus norvegicus*
- Sprague–Dawley (SD) rat: *Rattus norvegicus*

Shrews: *Sorex* and *Blarina* sp
Meadowvole: *Microtus* sp
Redback vole: *Clethryonomys gapper*
Non–rodents
Domestic dog: *Canis familiaris*
Domestic goat: *Capra hircus*
New Zealand rabbit: *Oryctolagus cuniculus*
Domestic pig: *Sus scrofa*
Domestic sheep: *Ovis aries*